PCT WORLD INTELLECTUAL PROP
International E

WO 9609062A1

INTERNATIONAL APPLICATION PUBLISHED UNDEI

(51) International Patent Classification 6 :		(11) International Publication Number: WO 96/09062
A61K 38/10, 38/16, 38/20, C07K 7/08, 14/00, 14/54	A1	(43) International Publication Date: 28 March 1996 (28.03.96)

(21) International Application Number: PCT/US95/12099

(22) International Filing Date: 22 September 1995 (22.09.95)

(30) Priority Data:

08/311,307	23 September 1994 (23.09.94)	US
08/311,380	23 September 1994 (23.09.94)	US

(71) Applicant *(for all designated States except US)*: UNIVERSITY OF NEBRASKA BOARD OF REGENTS [US/US]; Regents Hall, 3835 Holdrege Street, Lincoln, NE 68598 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: TALMADGE, James, E. [US/US]; 501 Martin Drive, Bellevue, NE 68005 (US).

(74) Agents: REED, Janet, E. et al.; Dann, Dorfman, Herrell and Skillman, Suite 720, 1601 Market Street, Philadelphia, PA 19103 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: POLYPEPTIDE AGONISTS AND ANTAGONISTS OF HUMAN INTERLEUKIN-8

(57) Abstract

Polypeptide analogs derived from Interleukin-8 are provided. In one embodiment, these reagents act as agonists of alpha chemokines and are useful as chemotherapeutic compounds for the treatment of neoplasms, including solid tumors and leukemias. They may also be efficacious in the treatment of infectious diseases mediated by microorganisms, such as bacteria, fungi and viruses. In another embodiment, these peptides analogs have antagonist activity for alpha chemokines and therefore may be used as anti-inflammatory agents, as well as in the treatment of autoimmune disease and various chronic inflammatory conditions such as rheumatoid arthritis and psoriasis.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

POLYPEPTIDE AGONISTS AND ANTAGONISTS OF HUMAN INTERLEUKIN-8

FIELD OF THE INVENTION

The present invention relates to polypeptide analogs of the human cytokine interleukin 8 that function as agonists or antagonists of interleukin 8 and other human alpha chemokines. The invention also provides methods of using these peptide analogs to augment or inhibit various biological responses associated with alpha chemokines.

BACKGROUND OF THE INVENTION

Interleukin 8 (IL-8) is a human cytokine that promotes the recruitment and activation of neutrophil leukocytes and represents one of several endogenous mediators of the acute inflammatory response. In the past it was variously termed neutrophil-activating factor, monocyte-derived neutrophil chemotactic factor, interleukin-8 (IL-8), and neutrophil-activating peptide-1, (NAP-1). The term IL-8 has gained the widest acceptance and will be used herein.

The most abundant naturally occurring form of the IL-8 monomer is a 72-residue protein apparently derived by processing of a 99-residue precursor. Other proteins with related sequences, including neutrophil-activating peptide-2 and GROα (with melanoma growth stimulatory activity), are IL-8 homologues which have neutrophil-activating properties.

IL-8 is a member of the chemokine superfamily that is divided into two distinct functional classes: alpha (α) and beta (β). The members of each class share an organizing primary sequence motif. The α members are distinguished by a C-X-C motif with the first two cysteines in the motif separated by an intervening residue. C-X-C chemokines are potent chemoattractants and activators for neutrophils, and are represented by IL-8. The β family chemokines have a C-C motif and are

equally potent chemoattractants and activators of monocytes. It appears that the two sides of the chemokine family have clearly defined functions: the C-X-C subfamilies cannot activate monocytes while the C-C subfamily has no effect on neutrophils. Although these two families of chemokines have similar three dimensional structures, they demonstrate fairly low sequence homology (30 to 35%). Proteins within the same family, such as platelet factor four (PF-4), are structurally related to IL-8 (35% sequence identity) but lack the N terminal ELR sequence (Glu-Leu-Arg) which has been shown by site directed mutagenesis to be critical for IL-8 activity. Thus, PF-4 has an entirely different profile of biological activity. Indeed, when the ELR sequence is added to the N-terminus of PF-4 it has been found that the modified protein has potent neutrophil activation and chemoattractant properties (Clark-Lewis et al., Biochemistry 90:3574-3577, 1993). However, this is not true for all of the chemokines since two of the proteins related to IL-8, γ interferon inducible protein (IP-10) and monocyte chemoattractant protein 1 (MCP-1) do not acquire neutrophil activating properties when the ELR structural determinants are added. Interestingly, when the E and the L of the ELR motif are removed from IL-8 the molecule acts as an antagonist for IL-8 (Moser, B., et al., J. Biol. Chem. 268:7125-7128, 1993).

In other studies by Clark-Lewis (Clark-Lewis, I., et al., J. Biol. Chem. 269:16075-16081, 1994) it was shown that conservative substitutions are accepted into the 10-22 region of IL-8, which is not the case for the ELR motif (residues 4-6). These investigators observed that the disulfide bridges and the 30-35 turn provide a structural scaffold for the NH-2 terminal region which includes a primary receptor binding site (ELR motif) and secondary binding and conformational determinants similar to those seen in residues 10 through 22. Other studies using mutants of IL-8 and melanoma growth stimulating

activity (MGSA) and recombinant IL-8 α/β receptors stably expressed in human cells demonstrated that there was a second site on the molecule responsible for binding. It appears that the carboxy terminus distal to amino acid 50 is not important in high affinity binding to the α receptor although both the amino and carboxy termini appear to be important for binding to the β receptor (Schraufstatter, I.S., et al., J. Immunol. 151:6418-6428, 1993). In summary, it appears that IL-8 posesses at least two and maybe three regions responsible for binding of IL-8 to its receptor. Further, the specific contact pharmacophore may vary depending upon whether or not the α or the β receptor is being examined.

Inflammation and autoimmune responses are initiated by leukocytes which migrate out of the microvasculature and into the extravascular space in response to chemoattractant molecules. These chemoattractants may be released from the host and include cytokines and activated complement components. Alternatively, they may be released from an invading organism (e.g., N-formylated peptides or MDP dipeptide). Once exposed to chemoattractants within the vasculature, the leukocytes become activated and capable of adhering to the endothelium, providing the first step in the development of inflammation. Stimulated neutrophils adhere to the endothelium of the microvasculature in response to a gradient of chemoattractants which direct the cells into the extravascular space toward the source of the chemoattractant. (Anderson et al., J. Clin. Invest. 74:536-551, 1984; Ley, K., et al., Blood 77:2553-2555, 1991; Paulson, J.C., Selectin/carbohydrate-mediated adhesion of leukocytes, Adhesion: Its Role in Inflammatory Disease, W. H. Freeman, 1992; Lasky, L.A., The homing receptor (LECAM 1/L-selectin), Adhesion: Its role in inflammatory disease, W. H. Freeman, 1992.)

Bevilacqua (Bevilacqua et al., J. Clin. Invest. 76:2003-2011, 1985) has demonstrated that cytokine and

endotoxin release stimulates the endothelium to become more adhesive for leukocytes. Subsequent observations (Buyon, J.P., et al., Clin. Immunol. Immunopathol. 46:141-149, 1988; Abramson, S.B., et al., Hosp. Pract. 23:45-56, 1988; Clark-Lewis, I. et al., supra, 1993); have suggested that the endothelium has a critical role in the events leading to the development of the inflammatory lesion. This model of inflammation suggests that leukocytes are directed to an inflamed locus by stimulated endothelium. After stimulation with cytokines or bacterial products, the endothelium arrests leukocytes as they traverse (roll along) the microvasculature near sites of inflammation. After being forced to stop in the microvasculature, the leukocytes are then activated to adhere more tightly to the endothelium and to migrate to the abluminal aspect of the vessel. The leukocyte, once it is out of the blood vessel, is then capable of following a gradient of chemoattractants toward the exciting pathogen.

Vascular endothelium, activated by stimulants such as IL-1, IL-8, TNF, or LPS, appears to play a pivotal role in this process through the production of pro-inflammatory substances, including chemoattractants and cytokines such as chemokines.

The inflammatory properties of IL-8 were initially demonstrated from a purified natural product injected intradermally into rabbits (Rampart, M. et al., Am. J. Path. 135 (1):21-25, 1989). More recently, neutralizing antibodies to human IL-8 were shown to have a protective effect in inflammatory lung injury in rats. This antibody blocked the glycogen-induced accumulation of neutrophils in rats and was protective against lung interdermal vascular injury induced by the disposition of IgG immune complexes. This latter model of injury has been shown to be E-selectin dependent. The protective effect of the neutralizing antibody correlated with reduced tissue accumulation of neutrophils as measured by

myeloperoxidase content. Preliminary nonhuman primate studies have confirmed the activity of IL-8 on hematological parameters. IL-8 was administered by both bolus and continuous infusion to baboons. This resulted in a rapid, transient and severe granulocytopenia followed by granulocytosis that persisted as IL-8 levels remained detectable within the circulation. Histopathological examination revealed a mild to moderate neutrophil margination in the lung, liver and spleen which was of greater severity in animals receiving the continuous infusion of IL-8.

High levels of intravascular IL-8 have been reported in systemic conditions such as septic shock (Danner, R.L., et al., Clin. Res. 38:352A, 1990). These authors have speculated that intervascular IL-8 may impair leukocyte adhesion and thus protect organs from PMN mediated injury. The intravenous administration of IL-8 induced an immediate and transient neutropenia that was similar in kinetic profile to that described with other chemoattractants. This neutropenia was a result of pulmonary PMN sequestration and is consistent with the demonstration of abundant IL-8 receptor on PMNs. Following this transient neutropenia, (approximately 30 minutes) cells recirculate with a normal half life. Shortly thereafter neutrophilia, a characteristic of IL-8, is observed. The neutrophilia likely reflects recruitment of mature PMNs to a marginal pool in the lung and other organs as well as immature PMNs from the marrow.

Endothelial cells can exert both proinflammatory and anti-inflammatory effects by virtue of the mediators they generate. Endothelial cells can be stimulated to generate IL-8, but unlike other mediators, IL-8 may be released from the endothelial cell. Alternatively, endothelial cell produced IL-8 is an important chemoattractant and activator of neutrophils. There is evidence that systemic IL-8 can bind to

endothelial cells, causing a local activation of the endothelium that results in the ability of this altered endothelium to attract neutrophils that have come into contact with the (activated) endothelium. One working hypothesis is that IL-8 initially functions as a proinflammatory cytokine, whereas its continued generation and release from the endothelium ultimately causes a down regulation of neutrophils, with a curtailment in their further recruitment. Whether the cell associated IL-8 or released IL-8 provides the vital contribution to the outcome of the inflammatory response remains unresolved.

IL-8 receptors are "promiscuous" and respond with a calcium flux when bound by structurally related ligands with the following order of potency: IL-8 > MGSA > NAP-2. The order of potency correlates with the effectiveness of these compounds in binding to neutrophils during competitive binding assays with the radio-labeled IL-8. C5a, a structurally related chemoattractant that is similar in size and charge to IL-8 and which has a receptor in the same family, does not activate the IL-8 receptor.

The *in vitro* effects of IL-8 on neutrophils are similar to those of other chemotactic agonists such as C5a and fMet-Leu-Phe and include induction of a transient rise in cytosolic free calcium, the release of granules containing degradative enzymes such as elastase, the respiratory H_2O_2 burst, neutrophil shape change, and chemotaxis. IL-8 appears to bind to at least one class of receptor sites on neutrophils with a frequency of approximately 64,000/cell and a K_d of 0.2 nM.

The three-dimensional structure of IL-8 is known by two-dimensional NMR and x-ray diffraction techniques. The IL-8 monomer has antiparallel β strands followed by a single overlying COOH-terminal α helix. Two disulfide bridges, between cysteines 7 and 34, and between cysteines 9 and 50 seem to stabilize the tertiary

structure. Residues 1-6 and the loop residues 7-18 seem to have little defined secondary structure. In solution, IL-8 is a noncovalent homodimer which is stabilized primarily by interactions between the β strands of the two monomers.

Examination of the three-dimensional structure indicates that following the cysteine at position 50, the residues form a type 1β turn (at residues 51 to 55) followed by an amphipathic α helix (at residues 55 to 72) that transverses the β sheet. The hydrophobic face of the α helix interacts with and stabilizes the hydrophobic face of the β sheet. Some of the interactions are between the two subunits of the dimeric molecule.

Interleukin-8 has shown both anti-tumor and anti-infective therapeutic activity. IL-8 has been shown to induce the regression of macroscopic tumors in a model of peritoneal carcinomatosis in the rat. In this model, IL-8 was shown to recruit PMN to the challenge site but did not enhance PMN infiltration of the tumor or the cytotoxic activity of PMN. Regardless, it did have significant therapeutic activity which may be secondary to PMN cytotoxicity and associated with other intermediate cells. It is suggested that lymphocytes could be involved since IL-8 has also demonstrated the ability to stimulate T-cell chemotaxis. (Lejeune, P., et al., Cancer Immunol. Immunotherapy. 38:167-170, 1994). Similarly, Interleukin-8 has shown therapeutic activity in nonneutropenic mice who received IL-8 shortly before challenge and at the site of infectious challenge with either *P. aeruginosa*, *Klebsiella-pneumoniae*, or *Plasmodium-berghei*. (Vogels, M.T., et al., Antimicrob-Agents-Chemother. 37:276-280, 1993).

In other antitumor studies IP-10, an alpha chemokine whose secretion is induced by IFN-γ and LPS, was genetically engineered into tumor cells. The expression of IP-10 by several tumor cell lines had no effect on the growth of these tumor cells *in vitro* but

elicited a powerful host mediated anti-tumor effect *in vivo*. Indeed, tumors genetically engineered to secrete IP-10 elicited a T-lymphocyte dependent anti-tumor response resulting in the rejection of tumors *in vivo*. Animals injected with these tumor cells do not develop tumors or develop tumors which spontaneously regress. Furthermore, administration of parental tumor cells admixed with IP-10 secreting tumor cells do not give rise to tumors in experimental animals. This suggests that in addition to being chemotactic for T-cells (Clark-Lewis, I., et al., supra, 1994) alpha chemokines may also act as T-cell adjuvants and therapeutics via T-cell chemotaxis and/or augmentation (Luster, A.D., J. Exp. Med. 178:1057-1064, 1993).

Because of its demonstrated anti-tumor and anti-infective activities, considerable interest exists in developing specific IL-8 agonists as immune adjuvants and anti-tumor agents for treatment of immunocompromised patients or patients with various forms of cancer. One strategy for developing agonists is to prepare polypeptide analogs which correspond to the known or suspected binding domains of naturally occurring IL-8 and related alpha chemokines. Such analogs are often found capable not only of binding to a cognate receptor, but of stimulating a desired biological response, such as anti-tumor activity for IL-8.

As it is established that IL-8 is also a key mediator of inflammatory diseases, it would also be desirable to identify substances capable of blocking or interrupting the activity of IL-8 for use in anti-inflammatory compositions. Such compositions may prove to be advantageous over presently available NSAID's, steroid based anti-inflammatory drugs and cytotoxic drugs which often have severe side-effects with the continued usage that is required for chronic inflammatory diseases. It would also be desirable to identify IL-8 analogs

having an increased inflammatory activity for medical research applications.

IL-8 has been previously produced through chemical synthesis (Clark-Lewis, et al., Biochemistry 30: 3128-3135, 1991) and by recombinant DNA methods (Herbert, et al., J. Biol. Chem. 286: 18989-18994, 1991). In addition, it is known that IL-8 exists in several forms that vary at the NH_2 -terminus, which have been detected in preparations purified from natural sources. These variations correspond to the predominant 72-residue form (which is generally considered to be the prototype IL-8 molecule); a 77-residue form having 5 additional NH_2 -terminus amino acids on each monomer; and, two shortened forms having residues 3-72 and 4-72 of the 72 amino acid form, respectively.

SUMMARY OF THE INVENTION

The present invention involves low molecular weight peptides which have agonistic or antagonistic activity for IL-8 or other alpha chemokines. The peptide agonists have an amino acid sequence with the following formula:

Glu-Leu-Arg-Cys-Xaa_1-Cys-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-Xaa_{12}

wherein

Xaa_1 is Gln, Met, or Val;

Xaa_2 is Ile, or Val;

Xaa_3 is Lys, Gln, or Ser;

Xaa_4 is Thr, or Ile;

Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;

Xaa_6 is Ser, Gln, Thr, or Ala;

Xaa_7 is Lys, Arg, or His;

Xaa_8 is absent or is Pro, Phe, or Gly;

Xaa_9 is absent or is Phe, Ile, or Val;

Xaa_{10} is absent or is His, Lys, or Arg;

Xaa_{11} is absent or is Pro, Leu, or Phe; and

Xaa_{12} is absent or is Lys, His or Arg.

More particularly, a preferred embodiment of the present invention comprises an amino acid sequence with agonist activity substantially equivalent to Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys as well as similar analogs having additional N-terminal amino acids. These polypeptides act as anti-cancer and anti-infective agents for the therapy of leukemia and solid tumors and metastatic disease. These embodiments also have anti-infective therapeutic activity for viral, bacterial, fungal, yeast and parasitic infections.

In other embodiments of the invention, the polypeptide is modified selectively to provide additional agonists of IL-8. In one particular embodiment, IL-8 agonists may include peptides truncated by 3 amino acid residues at the C terminus by deletion. In another particular embodiment, agonists for IL-8 or other alpha chemokines include cysteine substitutions by aminobutyric acid, homocysteine or diaminosuberic acid.

The inventor herein has investigated several low molecular weight peptide analogs of IL-8 with biological activity that substantially compete for IL-8 binding and are based on IL-8 sequences. Truncation of native IL-8, particularly in the C-terminal region thereof, yields IL-8 peptidal mimetics having the therapeutically useful properties of anti-neoplastic and anti-infective drugs. More particularly, the IL-8 low molecular weight peptide analogs of the present invention comprise an amino acid sequence with biological activity substantially equivalent to the IL-8 sequence beginning at residue 4 and continuing through residue 20, wherein at least the N-terminal residues found to be critical for neutrophil binding and stimulation, i.e., Glu-4, Leu-5, Arg-6 are contained at the N-terminal region.

In a further embodiment, IL-8 analogs include an additional two or three residues at the amino terminus of the 20 residue peptide so as to provide the 1-20 forms that are also useful as IL-8 agonists. In addition, significant biological activity is associated with low molecular weight peptide analogs from residue 4-17. The resulting in a 13 amino acid peptide also has agonist activity for IL-8.

Accordingly, this invention provides a biologically active human IL-8 low molecular weight peptide analog having an amino acid sequence substantially equivalent to the IL-8 polypeptide, beginning at residue 4 and continuing to residue 17 or 20.

In an alternative embodiment of the present invention, IL-8 peptide analogs with antagonistic activity have also been synthesized. These peptide analogs are comprised of an amino acid sequence substantially equivalent to those described above, except lacking the N-terminal pair of residues (i.e., Glu-Leu). The antagonist analogs compete strongly with IL-8 for neutrophil binding without signal transduction, due to the deletion of Glu-Leu (two-thirds of the "ELR" motif required for signal transduction activity).

In preferred embodiments, the peptide comprises the sequence: Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys as well as similar analogs having either one or two additional N-terminal amino acids. In another particular embodiment, antagonists that compete with IL-8 yet still retain antagonist properties are obtained by replacing residues Glu and Leu with non-polar amino acids, such as Ala, Val or Leu.

In other embodiments of the invention, the IL-8 sequence is truncated from residue 21 to the C-terminus as well at N-terminal residues 1 to 5. Thus the antagonist analogs which contain the N-terminal residues found to be critical for neutrophil binding and

stimulation, i.e., Arg 6, begin at residue 6 and continue through residue 20.

IL-8 analogs have also been synthesized that include an additional one or two non-polar residues at the amino terminus of the 14 residue peptide so as to provide the 4 or 5-20 forms that are also useful as IL-8 antagonists. In addition, significant biological activity is associated with low molecular weight peptide analog from residue 6-17 resulting in a 12 amino acid peptide with antagonist activity for IL-8.

Accordingly, this invention also provides a human interleukin-8 low molecular weight peptide analog with antagonist activity having an amino acid sequence substantially equivalent to those found at the N-termini of native IL-8, i.e., amino acids 6 through 20 or 6 through 17. Further, equal or greater activity is associated with the addition of Ala, Val, Leu or other amino acid at residue 4 and 5.

This invention also provides methods of use of these agonistic and antagonistic peptide analogs of human IL-8 both *in vivo* and *in vitro*.

In contrast to the claimed invention in PCT/CA92/00528 (WO 93/11159) which describes a 77 amino acid antagonist to IL-8, the embodiments of the present invention are related to low molecular weight peptide analogs of IL-8 which are less than 30, more preferably less than 20, and most preferably 11 to 17 amino acids in length. This shorter length allows for more rapid and extensive tissue distribution of the drug on administration as compared to a 70 amino acid therapeutic. Further, this invention substantially increases the purity of the product through reduced errors inherent to the manufacture of a large protein. In addition, this invention has a significant reduction in the cost of goods as compared to the invention in the aforementioned patent. Based on the homogeneity of the alpha chemokines, both sequence and three dimensional,

agonists and antagonists for both known and additional alpha chemokines can be derived based on the present invention. The present claims include information on the reduction to practice of an invention specific for the IL-8 ligand and receptor.

BRIEF DESCRIPTIONS OF THE DRAWINGS

Figure 1: FACS analysis profiles of Ca^+ release from polymorphonuclear monocytes (PMNs), unstimulated (Fig. 1A) or stimulated with native IL-8 (Fig. 1B).

Figure 2: FACS analysis profiles of Ca^+ release from untreated (Fig. 2A) and PBS treated (Fig. 2B) PMNs.

Figure 3: FACS analysis profiles of Ca^+ release from untreated (Fig. 3A) and analog 1 treated PMNs (Fig. 3B).

Figure 4: FACS analysis profiles of Ca^+ release from untreated PMNs (Fig. 4A) and PMNs treated with IL-8 and analog 12.

Figure 5: A graph of a dose response curve showing the chemotactic effects of a single i.p. injection of analog 1 in increasing concentrations. X axis represents the dose of analog 1 in μg/animal; Y axis depicts cell number x 10^6 in the peritoneum.

Figure 6: A graph of a dose response curve showing the chemotactic effects of a single i.p. injection of analog 1 in increasing concentrations. X axis represents the dose of analog 1 in μg/animal; Y axis depicts cell number x 10^6 in the peripheral blood.

Figure 7: A graph showing the different cell types present in the peritoneum in response to increasing amounts of i.p. injected analog 1. The X axis represents the dose of analog 1 in μg/animal; The Y axis represents cell number x 10^6 in the peritoneum. ● lymphocytes; ■ PMNs; ▲ monocytes.

Figure 8: A graph showing the absolute cell number of different cell types present in the peritoneum after an i.p. injection of analog 1. The X axis represents the dose of analog 1 in μg/animal; The Y axis represents cell number x 10^6 in the peritoneum. ● lymphocytes; ■ PMNs; ▲ monocytes.

Figure 9: A graph showing the different cell types present in the peripheral blood after a single i.p. injection of analog 1. The X axis represents the dose of analog 1 in μg/animal. The ratio of lymphocytes to PMNs is shown on axis Y1; the number of monocytes is shown on Y2. ● lymphocytes; ■ PMNs; ▲ monocytes.

Figure 10: A graph showing the absolute cell number of different cell types present in the peripheral blood after a single i.p. injection of analog 1. The X axis represents the dose of analog 1 in μg/animal. The ratio of lymphocytes to PMNs is shown on axis Y1; the number of monocytes is shown on Y2. ● lymphocytes; ■ PMNs; ▲ monocytes.

Figure 11: A graph showing the kinetics of the in vivo chemotactic response in the peritoneum following an i.p. injection of 10 μg of analog 1. The X axis represents time in hours and the Y axis represents the

absolute cell number. ● lymphocytes; ■ PMNs; ▲ monocytes.

Figure 12: A graph showing the effects of increasing doses of analog 12 on arachidonic acid induced ear inflammation in mice. The X axis represents increasing doses of analog 12 in μg/animal. The Y axis represents ear thickness measured in units of .001 inches.

Figure 13: Graphs of competitive binding assays showing the ability of peptide analogs to displace radiolabled IL-8 from the IL-8 receptor. The X axis represents increasing molar amounts of analog. The Y axis shows the % of total binding. The analogs tested are as follows: 13A XXR, Sequence I.D. No. 12; 13B CVC, Sequence I.D. No. 23; 13C CMC, Sequence I.D. No. 24; 13D FH, Sequence I.D. No. 25.

Figure 14: Graphs of competitive binding assays showing the ability of peptide analogs to displace radiolabled Groα from the IL-8 receptor. The X axis represents increasing molar amounts of analog. The Y axis shows the % of total binding. The analogs tested are as follows: 13A XXR, Sequence I.D. No. 12; 13B CVC, Sequence I.D. No. 23; 13C CMC, Sequence I.D. No. 24; 13D FH, Sequence I.D. No. 25.

DETAILED DESCRIPTION OF THE INVENTION

In order to provide an understanding of several of the terms used in the specification and claims, the following definitions are provided:

Biological activity - The term biological activity is a function or set of functions performed by a molecule in a biological context (i.e., in an organism or an *in vitro* surrogate or facsimile model). For IL-8 or other alpha chemokines, biological activity is

characterized by chemotactic activity (preferably PMNs but may also include T lymphocytes and/or monocytes/macrophages). It may also include second messenger and/or increases in elastase activity by PMNs.

A low molecular weight peptide analog - This identifies a natural or mutant (mutated) analog of a protein (generally less then 20-30 residues in length, comprising a linear or discontinuous series of fragments of that protein which may have one or more amino acids replaced with other amino acids and which has altered, enhanced or diminished biological activity when compared with the parent or non-mutated protein.

Substantially equivalent biological activity - is that profile of activity which defines IL-8 or other alpha chemokines. In *in vitro* surrogate models this may include chemotaxis of PMNs, T lymphocytes or monocytes; Ca^{++} signal transduction; or increases in elastase activity. *In vivo* this would be defined as the chemotaxis of PMNs to a localized site for example the peritoneum following i.p. injection or to the peripheral blood.

Substantially or essentially equivalent peptide analogs. An analog is essentially equivalent to another if it has one or more of the biological activities characteristic of human IL-8, essentially has the same number of amino acids as the analog specified in Tables 2 and 3 with no more than two additions or deletions and, in comparison with the sequence of the specified analog, has at most five amino acid substitutions, all of which would be considered neutral in the art (i.e., acidic for acidic, basic for basic, uncharged polar for uncharged polar, hydrophobic for hydrophobic, and the like).

The present invention provides composition comprising short polypeptide analogs of IL-8 or related alpha chemokines that bind to cellular chemokine receptors and act as agonists or antagonists of IL-8 or other alpha chemokines. These polypeptide analogs

generally correspond to the amino-terminal domain of IL-8 or related chemokines, as described in greater detail herein. The complete amino acid sequence of naturally-occurring IL-8 has been published (Clark-Lewis et al., J. Biol. Chem. 269: 16075-16078, 1994); the twenty amino acid residues comprising the amino-terminal domain is set forth below as Sequence I.D. No. 26 (the N-terminal Ser is residue number 1; the C-terminal Lys is residue number 20):

Ser-Ala-Lys-Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys.

It has been discovered in accordance with the present invention that short, amino-terminal peptide analogs of IL-8 and alpha chemokines are capable of binding to their cognate receptors and depending on the inclusion of the Glu-Leu-Arg ("ELR") domain at the amino terminus, can tranduce biological signals associated with native IL-8 and other alpha chemokines. Thus, peptide analogs of the present invention that contain the ELR domain can act as IL-8 agonists. It has also been discovered in accordance with the present invention that removal of the ELR domain from the peptides of the invention results in analogs capable of binding cognate receptors, but incapable of signal transduction. These analogs are useful as antagonists of IL-8 and other alpha chemokines. The overall sizes and structures of the analogs are similar except that the agonist group contains the intact ELR domain while the antagonist group is lacking most of the domain.

TABLE 1

The amino acids are identified in the present application according to the three-letter or one-letter abbreviations in the following Table 1:

Amino Acid	3-letter Abbreviation	1-letter Abbreviation
L-Alanine	Ala	A
L-Arginine	Arg	R
L-Asparagine	Asn	N
L-Aspartic Acid	Asp	D
L-Cysteine	Cys	C
L-Glutamine	Gln	Q
L-Glutamic Acid	Glu	E
Glycine	Gly	G
L-Histidine	His	H
L-Isoleucine	Ile	I
L-Leucine	Leu	L
L-Methionine	Met	M
L-Norleucine	NorLeu	J
L-Ornithine	Orn	O
L-Phenylalanine	Phe	F
L-Proline	Pro	P
L-Serine	Ser	S
L-Threonine	Thr	T
L-Tryptophan	Trp	W
L-Tyrosine	Tyr	Y
L-Valine	Val	V
L-Lysine	Lys	K
"Asx" means Asp or Asn		
"Glx" means Glu or Gln		

The present invention includes peptide sequences with agonist activity having the following formula:

Glu-Leu-Arg-Cys-Xaa_1-Cys-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-Xaa_{12}

wherein

Xaa_1 is Gln, Met, or Val;

Xaa_2 is Ile, or Val;

Xaa_3 is Lys, Gln, or Ser;

Xaa_4 is Thr, or Ile;

Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;

Xaa_6 is Ser, Gln, Thr, or Ala;

Xaa_7 is Lys, Arg, or His;

Xaa_8 is absent or is Pro, Phe, or Gly;

Xaa_9 is absent or is Phe, Ile, or Val;

Xaa_{10} is absent or is His, Lys, or Arg;

Xaa_{11} is absent or is Pro, Leu, or Phe; and

Xaa_{12} is absent or is Lys, His or Arg.

TABLE 2

Exemplary of the analogs of the present invention having agonist activity are the following:

Analog 1 (Sequence I.D. No. 1):

Derived from Alpha Chemokine - IL-8 (also called NAP-1)

Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 2 (Sequence I.D. No. 2):

Derived from Alpha Chemokine - IL-8 (also called NAP-1)

Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe

Analog 3 (Sequence I.D. No. 3):

Derived from Alpha Chemokine - GRO-α (also called MGSA)

Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Pro-Lys

Analog 4 (Sequence I.D. No. 4):

Derived from Alpha Chemokine - GRO-β (also called MIP-2σ)

Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Leu-Lys

Analog 5 (Sequence I.D. No. 5):

Derived from Alpha Chemokine - GRO

Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Met-Thr-Gly-Val-His-Leu-Lys

Analog 6 (Sequence I.D. No. 6):

Derived from Alpha Chemokine - GRO-γ (also called MIP-2β)

Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-His-Leu-Lys

Analog 7 (Sequence I.D. No. 7):

Derived from Alpha Chemokine-β thromboglobulin (also called NAP-2)

Glu-Leu-Arg-Cys-Met-Cys-Ile-Lys-Thr-Thr-Ser-Gly-Ile-His-Pro-Lys

Analog 8 (Sequence I.D. No. 8):

Derived from Alpha Chemokine - 9E3

Glu-Leu-Arg-Cys-Gln-Cys-Ile-Ser-Thr-His-Ser-Lys-Phe-Ile-His-Pro-Lys

Analog 9 (Sequence I.D. No. 9):

Derived from Alpha Chemokine - 310C

Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-His

Analog 10 (Sequence I.D. No. 10):

Derived from Alpha Chemokine - CNC

Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Val-Ala-Gly-Ile-His-Phe-Lys

Analog 11 (Sequence I.D. No. 11):

Derived from Alpha Chemokine - ENA-78

Glu-Leu-Arg-Cys-Val-Cys-Leu-Gln-Thr-Thr-Gln-Gly-Val-His-Pro-Lys

TABLE 3

Preferred embodiments of the peptides of the present invention with antagonist activity are presented in Table 3 which follows:

Analog 12 (Sequence I.D. No. 12):

Derived from Alpha Chemokine - IL-8 (also called NAP-1; this analog is sometimes referred to herein as "XXR")

Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 13 (Sequence I.D. No. 13):

Derived from Alpha Chemokine - IL-8 (also called NAP-1)

Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe

Analog 14 (Sequence I.D. No. 14):

Derived from Alpha Chemokine - GRO α (also called MGSA)

Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Pro-Lys

Analog 15 (Sequence I.D. No. 15):

Derived from Alpha Chemokine - GRO-β (also called MIP-2α)

Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Leu-Lys

Analog 16 (Sequence I.D. No. 16):

Derived from Alpha Chemokine - GRO

Arg-Cys-Gln-Cys-Leu-Gln-Thr-Met-Thr-Gly-Val-His-Leu-Lys

Analog 17 (Sequence I.D. No. 17):

Derived from Alpha Chemokine - GRO γ (also called MIP-2β)

Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-His-Leu-Lys

Analog 18 (Sequence I.D. No 18):

Derived from Alpha Chemokine-B thromboglobulin (also called NAP-2)

Arg-Cys-Met-Cys-Ile-Lys-Thr-Thr-Ser-Gly-Ile-His-Pro-Lys

Analog 19 (Sequence I.D. No. 19):

Derived from Alpha Chemokine - 9E3

Arg-Cys-Gln-Cys-Ile-Ser-Thr-His-Ser-Lys-Phe-Ile-His-Pro-Lys

Analog 20 (Sequence I.D. No. 20):

Derived from Alpha Chemokine - 310C

Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-His

Analog 21 (Sequence I.D. No. 21):

Derived from Alpha Chemokine - CNC

Arg-Cys-Gln-Cys-Leu-Gln-Thr-Val-Ala-Gly-Ile-His-Phe-Lys

Analog 22 (Sequence I.D. No. 22):

Derived from Alpha Chemokine - ENA-78

Arg-Cys-Val-Cys-Leu-Gln-Thr-Thr-Gln-Gly-Val-His-Pro-Lys

Analog 23 (Sequence I.D. No. 23):

Derived from IL-8 (this analog is sometimes referred to herein as "CVC")

Arg-Cys-Val-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 24 (Sequence I.D. No. 24):

Derived from Il-8 (this analog is sometimes referred to herein as "CMC")

Arg-Cys-Met-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 25 (Sequence I.D. No. 25):

Derived from IL-8 (this analog is sometimes referred to as "FH")

Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His

The present invention includes analogs that are essentially equivalent to any analog of the invention, as specified in Tables 2 and 3. As defined above, an analog is essentially equivalent to one specified above if it has one or more of the biological activities characteristic of human IL-8, essentially has the same number of amino acids as the analog specified in Tables 2 and 3 with no more than two additions or deletions and, in comparison with the sequence of the specified analog, has at most five amino acid substitutions, all of which would be considered neutral in the art (i.e., acidic for acidic, basic for basic, uncharged polar for uncharged polar, hydrophobic for hydrophobic, and the like).

The acidic amino acids are Asp, Glu and gammacarboxyglutamic acid. The basic amino acids are

Arg, Lys, His and Orn. The hydrophobic amino acids are Ala Ile, Leu, Met, Nor, Phe, Trp, Tyr, Val, t-butylglycine, norvaline, cyclohexylalanine, t-butylalanine, amino-4phenybutyric acid, beta-2-thienylalanine, p-bromophenylalanine, p-chlorophenylalanine, p-iodophenylalanine, p-nitrophenylalanine, 3.5-diiodotyrosine, phenylglycine, and napthylalanine. Uncharged polar amino acids are Asn, Gln, Ser, and Thr. Gly can be substituted for an uncharged polar or a hydrophobic amino acid, but substitutions with Pro are avoided because helical structures may be destabilized by such a significant effect on secondary structure of inserting a Pro in place of another amino acid.

Substitutions with Cys are specified and may include substitution with α-aminobutyric acid (Aba). This non-natural amino acid is suggested to cause a super-imposition with cysteine. Its ethyl side chain is closer to being isoteric than any of the naturally occurring non-polar amino acids. In addition, it is specified that Cys can be substituted with homocysteine or diaminosuberic acid thereby retaining charge and size conformation but eliminating potential homodimerization.

The chiral amino acids of the IL-8 analogs of the present invention have the L configuration.

Analog 1 (Sequence I.D. No. 1) was designed as a structural mimic of IL-8 from amino acid residues 4 to 20 (Clark-Lewis et al., 1994, supra). Analog 1 contains overall homology with the amino acid sequence of human IL-8.

Analog 2 (Sequence I.D. No. 2) was designated as a structural mimic of human IL-8 from amino acid residues 4 to 17. Analog 2, which has been C-terminally truncated has a slight loss of activity when compared to analog 1.

Analog 12 (Sequence I.D. No. 12) was designed as a structural mimic of IL-8 from amino acid residues 6

to 20. Analog 12 contains overall homology with the amino acid sequence of human IL-8.

Analogs 13 and 25 (Sequence I.D. Nos. 13 and 25) were designated as structural mimics of human IL-8 from amino acid residues 6 to 17 (or 18). Analog 13 has been C-terminal truncated causing a slight loss of activity when compared to analog 12.

An analog of the present invention can be made by exclusively solid phase techniques, by partial solid-phase techniques, by fragment condensation, by classical solution coupling, or, as long as the analog consists of only amino acids among the twenty naturally occurring amino acids corresponding to codons of the genetic code, by employing recombinant DNA techniques with bacteria, such as *E.coli* or *B.subtilis*; yeast, such as *S.cerevisiae* or *P.pastoris*; or insect or mammalian cells.

Methods of making a polypeptide of known sequence by recombinant DNA techniques are well-known in the art. See, e.g., U.S. Pat. No. 4,689,318, which is incorporated herein by reference.

Methods for chemical synthesis of polypeptides are also well-known in the art and, in this regard, reference is made, by way of illustration, to the following literature: Yamashino and Li, J. Am. Chem. Soc. 100:5174-5178, 1978; Stewart and Young, Solid Phase Peptide Synthesis (WH Freeman and Co. 1969); Brown et al., JCS Perkin I, 1983, 1161-1167; M. Bodanszky et al., Bioorg. Chem. 2:354-362, 1973; U.S. Pat. Nos. 4,689,318; 4,632,211; 4,237,046; 4,105,603; 3,842,067; and 3,862,925, all of which are incorporated herein by reference.

Preferred, automated, step-wise solid-phase methods for synthesis of peptides of the invention are provided in the examples below.

The IL-8 analogs encompassed by the present invention have one or more of the biological activities of naturally occurring IL-8, as described above, and, as

such, are useful therapeutically in one or more of the ways in which IL-8 is known to be useful, e.g., as an anti-infective for bacterial, fungal, viral, and or protozoan infections, as an anti-neoplastic for both leukemia and solid or metastatic disease.

The biological activity of an analog of the invention is determined by comparing the analog with naturally occurring IL-8.

The analogs of the invention are employed therapeutically, under the guidance of a physician for the treatment of leukemic or solid tumors or metastatic disease and infectious diseases including but not limited to viral, bacterial, fungal, yeast or parasitic. They may also be used for the treatment of anti-inflammatory and auto immune disease. Disorders in this group include SLE, GVHD, RA, IBD, asthma and psoriasis.

The dose and dosage regiment of an analog according to the invention that is suitable for administration to a particular patient can be determined by a physician considering the patient's age, sex, weight, general medical condition, and the specific condition and severity thereof for which the analog is being administered. The physician must also determine the route of administration of the analog; the pharmaceutical carrier with which the analog may be combined; and the analog's biological activity, relative to that of naturally occurring human IL-8, in the above-described assays.

Generally, injection (intavenous, subcutaneous or transmuscular) of 1-500 μmol of analog/kg body weight, by bolus injection, by infusion over a period of about 5 minutes to about 60 minutes, or by continuous infusion is sufficient for therapeutic efficacy. Aerosol inhalation of 0.1 or 2 mg of analog/kg body weight is also sufficient for efficacy.

Intravenous, subcutaneous or intramuscular administration, by bolus injection or continuous

infusion, is preferred for use of the analogs of the invention in treatment of neoplastic, infectious, autoimmune or inflammatory diseases.

The analogs of the invention, or a pharmaceutically acceptable salt thereof, can be combined, over a wide concentration range (e.g., 0.001 to 11.0 wt %) with any standard pharmaceutical carrier (e.g., physiological saline, THAM solution, or the like) to facilitate administration by any of various routes including intravenous, subcutaneous, intramuscular, oral, or intranasal, including by inhalation.

Pharmaceutically acceptable acid addition salts of the analogs of the invention can be prepared with any of a variety of inorganic or organic acids, such as for example, sulfuric, phosphoric, hydrochloric, hydrobromic, nitric, citric, succinic, acetic, benzoic and ascorbic. The analogs can, for example, be advantageously converted to the acetate salt by dissolution in an aqueous acetic acid solution (e.g., 10% solution) followed by lyophilization.

Pharmaceutical compositions containing a compound of the present invention as the active ingredient in intimate admixture with a pharmaceutical carrier can be prepared according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral or parenteral. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for

example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. For parenterals, the carrier will usually comprise sterile water, though other ingredients, for example, to aid solubility or for preservative purposes, may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed. The pharmaceutical compositions will generally contain dosage unit, e.g., tablet, capsule, powder, injection, teaspoonful and the like, from about 0.001 to about 10 mg/kg, and preferably from about 0.01 to about 0.1 mg/kg of the active ingredient.

The following examples are provided to further illustrate the present invention and are not intended to limit the invention beyond the limitations set forth in the appended claims.

EXAMPLE 1

Synthesis of IL-8 Peptide Analogs. Peptides were synthesized using solid-phase methodology, generally described by Merrifield (J. Amer. Chem. Soc. 85:2149, 1963) (see also Stewart and Young, supra) with various modifications described herein. Synthesis was carried out on an Applied Biosystems 431A automated peptide synthesizer (Applied Biosystems, Foster City, CA, USA).

Sequential assembly of a peptide analog is conducted from the carboxy-terminus, which is bonded to a solid-phase resin, to the amino terminus; the addition of amino acids to a peptide chain is automated after the attachment of the carboxy-terminal amino acid to the resin.

For peptides that have a carboxyl group at the carboxy-terminus, p-chloromethyl-derivatized polystyrene

supports are employed, and the carboxy-terminal amino acid is esterified to the support via reaction with KF. Analogs with a C-terminal proline or a penultimate C-terminal proline may be synthesized using a 2-chlorotrityl chloride derivatized resin. Attachment of FMOC amino acids to the resin can be quantitated by spectrophotometric determination at 266 nm following treatment of a weighed sample with 50% piperidine in DMF. Substitution levels for automated syntheses are preferably between 0.2 and 0.6 mmol amino acid per g resin. A typical FMOC synthesis is performed on a scale of 0.1-0.25 mmol and thus is initiated with 0.15-1.25 g amino acid-derivatized resin. The 9-Fluorenyl methoxycarbonyl (FMOC) group was used for protection of the alpha amine group of all amino acids employed in the syntheses; however, other protecting groups known in the art for alpha amines can be employed successfully. Side-chain functionalities were protected as follows: Arg with 4-Phenylazobenzyloxycarbonyl; Cys, Gln, and His with trityl; Glu, Ser, and Thr with benzyl; Lys with tertiary butyloxycarbonyl; and Tyr with tertiary butyl.

Resins employed in the syntheses were purchased with the C-terminal residue already attached to derivatized polystyrene-1% divinyl-benzene (200-400 mesh), either by 4-hydroxymethylphenoxyacetic acid or 2-chlorotrityl chloride.

Steps in the syntheses of the IL-8 analogs employed the following Protocol I(a):

Step	Reagent	Mix Time (min)	# of Times
1	20% piperidine in NMP	16.4	2
2	0.45M HBTU/HOBT in DMF/ in NMP	7.6	1
3	NMP	4.6	1
4	2.0M DIEA in NMP	2.2	1
5	NMP	22.2	1
6	0.5M acetic anhydride/0.125M DIEA/0.015M HOBT/in NMP	6.4	1
7	NMP	4.6	1
8	Stop or return to step 1 for next coupling		

NMP = N-Methylpyrrolidone
HBTU = 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBT = 1-Hydroxybenzotriazole
DMF = Dimethylformamide
DIEA = Diisopropylethylamine

All chemicals were reagent or peptide synthesis grade and were used as purchased. DIEA and NMP were from Fisher Scientific, Fair Lawn, NJ, USA. Piperidine, HOBT and HBTU were from Advanced ChemTech, Louisville, KY, USA. DMF was from E. Merck, Gibbstown, NJ, USA,. Acetic anhydride was from FLUKA, Buchs, Switzerland. The coupling of amino acids was carried out for 45 minutes with a four-fold excess of the activated esters of the FMOC amino acids with respect to the available amine sites on the resin.

After assembly of the completed analog, the amino-terminal FMOC group was removed using step 1 of the above protocol and then the resin was washed with methylene chloride and dried. The analogs were then deprotected and removed from the resin support by treatment with TFA for 2 hours at 25 °C. The peptide was precipitated with cold diethyl ether, the liquid phase was filtered away and the peptide was extracted with 10% acetic acid in water and lyophilized.

The resulting crude preparations were purified by preparative high performance liquid chromatography (HPLC) on a Waters C-18 column (40 x 200 mm) (Millipore Corp, Millford, MA, USA) and analyzed by analytical HPLC. Preparative HPLC separations were performed with the Waters column on a Waters Delta Prep 4000 System (Millipore Corp, Millford, MA, USA) at a flow rate of 75 ml/min. Samples were introduced in 0.1% TFA (running buffer) and after a 5 minute lag to ensure complete loading, eluted from the column with a 1%/minute acetonitrile gradient with an elution time of 24-26 minutes. Peptide fractions were monitored by UV absorbance at 220 nm. In all cases, fractions were manually collected at peak detection. The purified fractions were analyzed on an analytical HPLC System, Waters 712 WISP, using a Waters C-18 column (8 x 100 mm) using 0.1% TFA and an acetonitrile gradient. Other HPLC buffer systems which may be employed in the analytical HPLC include triethylamine phosphate (TEAP), pH 2.5-3.0, TEAP, pH 6.5, and a mixture of 0.1% phosphoric acid, 0.1M sodium perchlorate, pH 2.5 with an acetonitrile gradient. Although the TFA buffer system does not resolve microheterogeneous contaminants as well as other systems, recovery is generally 50-90% higher. A portion of a fraction from the preparative HPLC which appeared homogenous by analytical HPLC was removed, lyophilized and hydrolyzed for amino acid analysis. These portions with the proper amino acid compositions and proper mass,

determined by matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry, were treated to replace the TFA with acetic acid and were then subjected to bioassay.

For amino acid analysis, a sample of analog was hydrolyzed in 6N HCl containing 1% phenol for 70 minutes at 110 °C. Analyses were performed by a procedure which is a modification of the method of Cohen and Michaud (Anal. Biochem 211:279-287, 1993) employing the AccQ Tag reagent (Waters, Millford, MA, USA).

The synthesis of Analog 1 (Sequence I.D. No. 1) and Analog 12 (Sequence I.D. No. 12), both, peptides based on the IL-8 amino terminal domain, was initiated by using 581 mg of an H-LYS(BOC) 2-chlorotrityl resin (substitution level = 0.43 mmol/g), purchased from AnaSpec, Inc., (San Jose, CA, USA). The synthesis was carried out automatically by the ABI 431A Peptide Synthesizer. The amount of components is summarized in the following Table.

Table 4.

CYCLE	Grams/protected amino acid
1	.337 P
2	.620 H
3	.387 F
4	.337 P
5	.469 K
6	.327 S
7	.494 Y
8	.341 T
9	.469 K
10	.353 I
11	.586 C
12	.612 Q
13	.586 C
14	.397 R

Upon completion of the synthesis, 1.2 g of Analog 1 peptide-resin and 0.8 g of Analog 12 peptide-resin were obtained. These were added to 10 ml of TFA in two 100 ml round bottom flasks with 500 μl of H_20 500 μl thioanisole, 250 μl ethanedithiol and 750 mg phenol and stirred at 25 °C for 2 hours. The reactions were terminated by the addition of 100 ml cold diethyl ether to precipitate the peptides. The solution was filtered and the peptides were extracted with 100 ml 10% acetic acid in water, frozen, and lyophilized. A small portion of each was dissolved in 225 μl of 0.1% TFA and injected onto the analytical HPLC using the previously described

conditions to determine elution characteristics for the preparative scale purification. The remainder of the crude peptide was loaded onto the preparative system and eluted under the above conditions. A 0-40% acetonitrile gradient over 40 minutes was used to elute the peptide components. An aliquot of homogeneous fractions from preparative HPLC was removed, and then hydrolyzed for amino acid analysis. Amino acid analysis results for Analog 1 (Sequence I.D. No. 1) preparation were as follows: Ser (1)0.96, Glx (2)1.98, His (1)1.04, Thr (1)0.94, Arg (1)1.08, Pro (2)1.82, Cys (2)1.86, Tyr (1)1.07, Lys (3)2.94, Ile (1)1.13, Phe (1)1.14, and Leu (1)0.98.. The MALDI-TOF mass spectrum showed the proper mass peak and only peptide-matrix peaks. Amino acid analysis of a weighed sample showed the powder to be 85.9% peptide by mass.

Amino acid analysis results for Analog 12 (Sequence I.D. No. 12) preparation were as follows: Ser (1)0.96, Glx (1)0.96, His (1)1.04, Thr (1)0.94, Arg (1)1.08, Pro (2)1.82, Cys (2)1.86, Tyr (1)1.07, Lys (3)2.94, Ile (1)1.13, Phe (1)1.14. The MALDI-TOF mass spectrum showed the proper mass peak and only peptide-matrix peaks. Amino acid analysis of a weighed sample showed the powder to be 88.1% peptide by mass.

EXAMPLE II

Administration of IL-8 Peptide Analogs affects Ca^+ Mobilization in PMNs. Polymorphonuclear leukocytes (≥95% neutrophils, PMNs) were isolated from heparinized (10 U/ml) blood collected from healthy volunteers. Erythrocytes were removed by 6% dextran sedimentation for 30 minutes at room temperature, and then the supernatant was subjected to Ficoll (Ficoll-Paque, Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation. PMNs were obtained from the pellet. Contaminated erythrocytes were eliminated by one cycle of hypotonic lysis. After an additional washing step, the cells were counted and

adjusted to a final concentration of $5x10^6$/ml in RPMI 1640 (Gibco, Grand Island, NY).

Fluo-3 Loading - The loading procedure with Fluo-3 (Molecular Probes, Eugene, OR) was carried out with PMNs suspended at a density of $5x10^6$/ml in fresh RPMI 1640 containing 2.0 μM Fluo-3 AM (prediluted in dimethyl sulfoxide; Sigma Chemicals) in polypropylene tubes (Falcon 2063, Becton Dickinson). PMNs were incubated for 25 minutes at 37 °C in a 5% CO_2 incubator. During the incubation period, the PMNs were gently agitated twice. To remove extracellular Fluo-3 AM, cells were washed twice with RPMI 1640 and with Ca^{2+} and Mg^{2+} - free phosphate-buffered saline (PBS) containing 100 mM KCl and 5 mM HEPES buffer at pH 7.05. Finally, the cells were adjusted to a density of $5x10^5$/100 μl in the PBS buffer and resuspended in 5 x 35 mm tubes. The samples were kept in the dark at room temperature until use.

Measurement of $[Ca^{2+}]$ in Flow Cytometry - Before addition of the stimuli the fluorescence channel of the FACScan Plus (Becton Dickinson) was adjusted to find the basal fluorescence level of loaded but unstimulated PMNs. The samples were excited by an argon laser at 488 nm and emission was measured at 525 nm (green fluorescence, channel FL1). The temperature has no effect on the appearance of neutrophil subpopulations with different $[Ca^{2+}]$ mobilizations. However, the experiments were carried out at room temperature, 22°C. PMNs were then stimulated at different concentrations with full-length IL-8/NAP-1 or experimental polypeptide analogs. The events were acquired before and 5 s after addition of the stimuli, and acquisition was continued over 80 s. For each acquisition 2500-3000 events were collected in 6 s interrupted for a time interval of 8 s or less using the FACScan software (Becton Dickinson). The fluorescence of Fluo-3-loaded cells was measured in arbitrary fluorescence units of mean channel fluorescence (channel FL1). To control the influence of the medium, PBS

(supplemented as described above) was added instead of stimuli. A continuous measurement of unstimulated PMNs was also carried out. Both procedures did not result in an increase in arbitrary fluorescence units.

Analog 1 (Sequence I.D. No. 1) exhibits agonist activity on PMNs: Prior studies of IL-8/NAP-1 have demonstrated that this cytokine induces an increase in Ca^+ after stimulation of neutrophils. Figure 1B demonstrates the effect of the 77 amino acid IL-8 at 10^{-6}M on neutrophil Ca^+ release as measured and described above. Figure 1A shows unstimulated neutrophils. Figure 2A again shows unstimulated neutrophils whereas Figure 2B shows the lack of effects by the excipient alone. Treatment with Analog 1 induced a change in neutrophil Ca^+ concentration as shown in Figure 3B relative to untreated cells in Figure 3A. The data show that the majority of neutrophils showed an essentially identical increase in calcium concentration upon addition of either IL-8/NAP-1 or Analog 1.

Analog 12 (Sequence I.D. No. 12) exhibits antagonist activity on PMNs: As shown in figure 1B, treatment of neutrophils with native IL-8/NAP-1 leads to an increase of arbitrary fluorescence units as compared to excipient treated Fluo-3 loaded neutrophils (figure 2B). However, as shown in Figure 4B, when the PMN's were pre-incubated with both native IL-8 and analog 12 at 10^{-6} M, analog 12 blocked the appearance of the Ca^+ signal induced by IL-8 as shown in Figure 1B.

EXAMPLE III

***In vivo* Bioreactivity Assay of IL-8 Analogs with Agonist Activity.** A stock solution of 5 mg/ml of Analog 1 was prepared using Dulbelcco's phosphate buffered saline D-PBS. The solution was made 5x to compensate for the injection of 0.2 ml intraperitoneally (i.p). The mice were treated at various times with various doses of Analog 1 (Sequence I.D. No. 1). In

addition, control animals were injected with excipient only.

The mice used for the study were C57BL/6 females, which were obtained from Jackson Laboratories. The animals were 8-10 weeks of age and weighed 15-18g. The mice were housed in conventional cages, fed pelleted food and given water *ad libitum*.

At various times following injection, the mice were bled from the retinal orbital plexus and the blood (40 μl) was collected into heparinized capillary tubes. The blood was then analyzed using a Serona Baker 9000 Blood analyzer. In addition, blood films were made and differentials undertaken on at least 200 cells/sample.

The mice were sacrificed by ether overdose, the ventral skin reflected and the peritoneum lavaged with three 5 ml lavages of calcium-, magnesium-free HBSS. At one, two, four, eight and 24 hours, following injection, three to five mice and/or control mice were lavaged and the cells collected in 15 ml tubes. The peritoneal cells were then centrifuged and counted. In addition, a differential was done on the peritoneal cells in a cytospin preparation. For the cytospin, 25 μl of bovine albumin (Sigma Lot #70H0183) was first placed in the bottom of the chamber, and 100 μl of cell suspension (100,000 cells) added. The chambers were spun at 1000 rpm for 5 minutes and left to dry. They were then placed in leukostat fixative solution for 5 seconds followed by 45 seconds in Leukostat solution 1 and 45 seconds in Leukostat solution 2. Fixed cells were washed in deionized H_20 for 5 seconds and allowed to dry. The slides were analyzed under a microscope for differentials.

Analog 1 is an agonist with biological activity in *in vivo* assays. The *in vivo* chemotactic activity for cells into both the peritoneum and PBL are shown in this data set. Figure 5 shows the effect of a single i.p. injection of Analog 1 on peritoneal cellularity when

employed in increasing concentrations from 0.1-500 μg/animal. Optimal activity was observed at 10 μg/animal. Similarly, a significant increase in PBL cellularity (Figure 6) was observed with maximal activity at 0.1 and 10 μg/animal. The primary cell type elicited in the peritoneum by the i.p. injection of Analog 1 was PMNs as shown in Figure 7. Treatment with Analog 1 resulted in a four-fold increase in PMNs in the peritoneum and an almost two-fold increase in monocyte/macrophages (Figure 8). Similarly, there was an increase in the frequency of PMNs in the peripheral blood (Figure 9) with a concomitant significant increase in the absolute number of PMNs and monocytes in the peripheral blood (Figure 10).

Analog 1, having residues 4-20 of human IL-8, was synthesized according to the method described herein (Example 1). Figure 11 shows the kinetics of the *in vivo* chemotactic response in the peritoneum following i.p. injection of 10 μg of this peptidal low molecular weight analog. As mentioned previously, the greatest increase observed was in the number of macrophages and PMNs in the peritoneum.

These results are substantially identical to nonhuman primate studies which demonstrated the hematologic effects of native IL-8 following parenteral administration. IL-8 was administered by both push and continuous infusion to baboons. This resulted in a granulocytosis which persisted as long as IL-8 remained detectable within the circulation (Van Zee, K.J. et al., J. Immunol. 148:1746-1752, 1992). Similar results have been observed in rabbits where natural IL-8 was injected intradermally in the presence of a vasodilator substance. In those studies, IL-8 induced a neutrophil accumulation that was fast in onset, relatively short in duration and was associated with a parallel time course of plasma protein extravasation (Rampart, M., Am. J. Pathol. 135:21-25, 1989). Thus, the polypeptide Analog 1 of the

present invention elicits essentially the same biological responses *in vivo* as naturally-occurring IL-8.

EXAMPLE IV

***In vivo* Bioassay of IL-8 Analogs with Antagonist Activity.** A stock solution of 5 mg/ml of Analog 12 (Sequence I.D. No. 12) was prepared in D-PBS. Analog 12 consists of 88.1% active peptide and the calculations were adjusted to compensate for the activity of Analog 12. Serial dilutions were then made resulting in solutions of appropriate doses of analog 12 in 0.2 ml. The animals were injected intraperitoneally with 0.2 ml of the appropriate solution 1 hour prior to arachidonic acid application.

The study consisted of various dosage groups as well as excipient groups as contols.

The animals used for the study were C57BL/6 females, which were obtained from Jackson Laboratories (age 8 weeks and weight 15-18g). The mice were housed in conventional cages, fed pelleted food and given water *ad libitum*.

The arachidonic acid (Sigma Lot #42H7817) was dissolved in methyl alcohol resulting in a final concentration of 2 mg/20μl. At time zero, 10 μl of arachidonic acid was applied to each side of the test ear using a pipettor. Each mouse received a total of 2 mg and a total volume of 20 μl. One hour post challenge, the test and control ears were measured for thickness using a Mitutoyo No. 7300 gauge caliper. The thickness was recorded in units of 0.001 inches. To determine the total edema, the measurement of the control ear was subtracted from the test ear measurement (arachidonic acid ear).

Analog 12 is an antagonist with *in vivo* bioactivity. The effect of Analog 12 on arachidonic acid-induced ear inflammation was evaluated *in vivo*. In this model, the application of arachidonic acid to the

pinna of mice induces an immediate PMN-associated erythema and edema which may be measured by change in thickness. As shown in Figure 11, the administration of Analog 12 blocked edema with an approximate EC-50 of 1 μg/animal. In other recent studies in our laboratory similar suppression of arachidonic acid induced inflammation in the mouse pinna was observed with 100 mg/kg phenidone (NSAID).

These results are substantially the same as these reported in International Application No. PCT/CA92/00528. In that study, N-terminally truncated natural IL-8 (70 amino acids) was shown to inhibit inflammation using the rabbit plasma exudate dermal assay reported by Beaubien et al. (Biochem. J., 1990, 271:801). In that model, anti-inflammatory activity is monitored by a reduced edema formation and neutrophil accumulation in rabbit skin. In contrast to the large IL-8 analog described in PCT/CA92/00528, Analog 12 described in this example is a 14 amino acid IL-8 analog. The analog described in the aforementioned patent is a much longer antagonist which comprises approximately 45 amino acids of IL-8. These analogs require extensive synthesis and/or recombinant production which substantially increases both the cost of the goods and the probability of synthetic errors during manufacturing.

EXAMPLE V

Chemokines form a superfamily that is divided into two distinct functional classes: alpha and beta. All the members of each class share an organizing primary sequence motif. Alpha family members are distinguished by the C-X-C motif - where the first two cysteines of the motif are separated by an intervening residue. C-X-C chemokines are potent chemoattractants and activators for neutrophils. The beta family chemokines have a C-C motif and are equally potent chemoattractants and activators of monocytes. However, other roles are rapidly emerging for

chemokines as well. Thus, both alpha and beta chemokines, have been shown to attract both memory T-cell and eosinophils as well as trigger the release of histamine from basophils.

Edgington, discussed (Edgington et al., Biotech. 11:676, 1993) the potential to design site specific inhibitors and/or agonists for the chemokines. He states that most researchers believe that the greatest promise lies in creating small molecular peptides or mimetics that will compete with natural molecules for the chemokine binding sites. Because both the ligand and the receptor binding site are relatively small, drug designers have focused on understanding where the critical contact points lie that turn on the receptor signal transduction machinery. Many of these investigators believe that similarities in sequence will also yield structural similarities. Dr. Daniel Witt of Repligen, Cambridge, MA is quoted in the article as saying "If you have 15 different receptors and you know the crystal structure for two you can expect the structural model will hold true for all." It was suggested that both chimeric ligands and receptors should fold properly and yield data about critical contact points. "As a system, it is about everything you could ask for imposing structural-functional questions" i.e., there is strong homology amongst the receptors and ligands of the chemokines which will facilitate rational drug design. It is clear that existing drugs have been synthesized which target similar membrane spanning receptors including: histamine inhibitors, beta blockers, and serotonin receptors which have all yielded receptor antagonists to date.

"Receptor promiscuity" presents one problem to drug design of the chemokines. Within chemokine families, individual receptors bind multiple ligands. Determining chemokine structure and function after dispensing with the dogma of "one ligand, one receptor"

is extremely difficult, especially when target cells contain an ensemble of receptors on their membrane. For example, there are at least three kinds of chemokine receptors involved in binding the beta chemokines MCP-1, MIP-1α/β and RANTES. Based on studies to date, one receptor binds all three ligands, another binds only MIP-1α/β and a third only binds MCP-1. Similar observations of receptor/ligand promiscuity are seen with the alpha chemokines. High affinity binding to neutrophils is observed for IL-8; however, evidence for high and low affinity binding receptors is observed with NAP-2, ENA-78 and MGSA. Two closely related neutrophil derived IL-8 receptors, type A and type B, have been cloned whose binding characteristics could account for the binding observed with neutrophils: Type A receptors have a high affinity for IL-8 and low affinity for MGSA and NAP-2. The type B receptors bind IL-8 and MGSA with high and NAP-2 with intermediate affinity. However, it remains possible that more than two IL-8 receptors exist and are expressed on neutrophils. DNA sequence data and X-ray diffraction data will facilitate rational drug design. Based on this knowledge, efficacious peptide analogs can be synthesized and assayed in the assays specified herein.

EXAMPLE VI

Platelet factor 4 (PF-4) is carried within the alpha granules of platelets in the form of a non-covalent complex. PF-4 is a tetramer of four identical polypeptide chains, each of which contains 70 residues in the human moieties. When released into the plasma from activated platelets, PF-4 attracts white blood cells i.e., neutrophils and monocytes and it's release may be a signal that is involved in inflammation. Other possible functions of PF-4 derive from a strong binding to negatively charged polysaccharides especially heparan and heparin for which it has a dissociation constant of $10^{-7.5}$.

The ability of PF-4 to neutralize heparin and related polymers is of general interest because heparin has been shown to interact with over 50 different enzymes to suppress muscle growth and to accelerate angiogenesis in solid tumors. PF-4 has recently been patented as Oncostatin A for its ability to inhibit tumor growth. It has also been shown to reverse immunosuppression in mice.

PF-4 also binds tightly and preferentially to double stranded DNA *in vitro*. This binding probably does not occur *in vivo* but it might be an important attribute of several recently discovered growth related proteins which are homologous to PF-4. Members of this class are 1) induced by IFN-γ, 2) constitutively overexpressed in Chinese hamster and human cell lines, 3) strongly induced by Rous sarcoma virus and fibroblast cells and 4) over expressed in stimulated leukocytes.

In contrast to many of the other alpha chemokines, PF-4 does not induce comparable neutrophil responses although chemotaxis and exocytosis have been reported with concentrations that were 1,000 to 10,000-fold higher than those required for IL-8.

PF-4 crystallizes as a tetramer, although the monomer structure is similar to that of IL-8. In solution, human PF-4 is in equilibrium among monomers, dimers, and tetramers. PF-4 and IL-8 share 35% sequence identity, including the four cysteines. Molecular modeling studies suggest that a similar folding pattern will be found for all members of the C-X-C family. Even MCP which belongs to the C-C family (beta chemokine), has been found to have the same tertiary structure. Thus, it will be possible to sequence an agonist for PF4 in accordance with the present invention.

EXAMPLE VII

Platelet basic protein (PBP) is a highly specific platelet alpha granular protein that is a precursor of low affinity platelet factor 4 (LA-PF4) and

beta thromboglobulin (βTG). These proteins differ only in amino terminal amino acid sequence and isoelectric point. PBP is synthesized by megakaryocytes, contains 94 amino acids and is converted to LA-PF4 which contains 85 amino acid residues within megakaryocyte and platelet granules.

βTG itself (81 amino acid residues) as originally described cannot be detected in cell lysates prepared using trichloroacetic acid. It probably results from amino terminal cleavage of LA-PF4 or PBP after cell secretion. Production of βTG from PBP and LA-PF4 can be demonstrated *in vitro* by incubating the platelet release supernatant at 37°C or by limited cleavage with plasmin or trypsin. The three forms of βTG antigen are immunologically identical when using rabbit polyclonal antibodies. The biological activities of PBP and it's derivatives are not well understood. It has been proposed that LA-PF4 [also referred to as connective tissue activating peptide III (CTAP-III)] is a weak mitogen for connective tissue fibroblasts. It has also been reported that βTG antigen promotes chemotaxis in fibroblasts. In recent studies, it was observed that a cleavage product of βTG called neutrophil activating peptide (NAP-2) was formed in cultured and stimulated mononuclear cells and is a potent activator of human neutrophils. NAP-2 is a 78 amino acid peptide corresponding to the major carboxy-terminal fragment of βTG. It has 46% homology with NAP-1/IL-8. NAP-2 behaves as a typical chemotactic receptor agonist, inducing cytosolic free Ca^+ changes, chemotaxis, and exocytosis while PBB, LA-PF4 and PF4 have little such activity. It should be noted, as discussed above, that NAP-2 also interacts with the NAP-1/IL-8 receptors. Thus, in accordance with the present invention, it should be possible to synthesize agonists for NAP-2, including those that bind to the IL-8 beta receptor.

EXAMPLE VIII

IP-10 was originally isolated as a predominant messenger RNA form induced by IFN-γ or LPS in monocytes and its expression has been detected *in vivo* during the development of a delayed type hypersensitivity cellular immune response by monocytes, endothelial cells, and infiltrating mononuclear cells. In addition, IP-10 expression has been seen in the epidermis, dermis and cutaneous lesions of psoriasis, tuberculoid leprosy, and fixed drug eruptions.

IP-10 is a member of the chemokine superfamily and is approximately 30% homologous to IL-8 and PF-4. Recent studies have shown that IP-10 can elicit an anti-tumor inflammatory response that is capable of inhibiting the growth of plasmacytoma and mammary adenocarcinoma in immunocompetent mice. This effect is thymus dependent suggesting that IP-10 might act on T-cells. In addition, a neutrophil, and monocytic accumulation is seen as a result of IP-10 expression in immunocompetent but not nude mice. IP-10 does not have an ELR motif. ELR incorporation into IP-10 is not sufficient for IL-8 receptor interaction or neutrophil activation and suggests that IP-10 has a different receptor ligand conformation. However, hybrids formed between IL-8 and IP-10 could be designed which demonstrate that essential receptor binding motifs from the IL-8 sequence could be structured within the IP-10 molecule allowing IL-8 binding.

Recent reports have shown that IP-10 is expressed by activated but not by resting T-hybridoma cells, normal T-cells and thymocytes. While resting lymphocytes did not synthesize IP-10, a high level of IP-10 transcripts are found in lymphoid organs (spleen, thymus, and lymph nodes). Thymic and splenic stromal cells constitutively express high levels of both IP-10 messenger RNA and protein accounting for the high level of spontaneous expression in lymphoid tissue. Therefore,

in addition to its role as a pro-inflammatory cytokine, IP-10 may participate in T-cell effector function of perhaps T-cell development. IP-10 expression has also been shown to be induced in delayed type contact hypersensitivity in sensitized animals. Thus, in accordance with present invention, we will be able to synthesize agonists for IP-10.

EXAMPLE IX

Human tumors can constitutively express cytokines and growth factors. Melanoma cells constitutively express GRO-α, which is also termed "melanoma growth stimulatory" activity. Similarly, GRO-α is expressed in human colon tumors along with GRO-β and GRO-γ. These three genes, GRO-α, GRO-β, and GRO-γ, are closely linked on chromosome 4. GRO-β and GRO-γ show 90 and 86% sequence homology with GRO-α. The GRO-α/MGSA alpha chemokine has potent chemotactic, growth regulatory and transformative functions. The function of GRO-β and GRO-γ is unknown. GRO-α messenger RNA is selectively overexpressed in psoriasic epidermis and is reduced by therapy with Cyclosporin-A. It has been suggested that this over expression is a karyotinocyte response to activated T-cells in psoriasis. GRO-α/MGSA has been localized in a variety of cutaneous lesions. A raised level of immunoreactive GRO-αMGSA in diseased epidermis is detected in verruca vulgaris followed by psoriasis, keratoacanthoma, and squamous cell carcinoma. Detection of GRO-α in basal cell carcinoma is variably present in the sclerosis variant and absent in the more common nodular variant. Thus, in accordance with the present invention, we will be able to synthesize agonists for GRO-α, β, and γ.

EXAMPLE X

Recently, another alpha chemokine has been discovered and has been called ENA-78 (epithelial cell

derived neutrophil activator). ENA-78 shows significant amino acid sequence homology with NAP-2 (53%), GRO-α (52%), and IL-8 (22%). ENA-78 appears to activate neutrophils through the IL-8 receptor. ENA-78 has been cloned in pigs and was initially described as alveolar macrophage derived chemotactic factor 2. It shares 53% sequence homology with human NAP-2 and 61% sequence homology with the GRO-related proteins. It also has 67% sequence homology with the 78 amino acid ENA-78 and is felt to represent the porcine variant thereof. ENA-78 was initially identified in the conditioned medium of stimulated human epithelial cell line A549. It is produced in response to stimulation with either IL-1β or TNF-α and is produced and secreted concomitantly with IL-8, GRO-α, and GRO-γ. ENA-78 consists of 78 amino acids and has a molecular weight of 8357. The four cysteines are positioned identically to those of IL-8 and similar analogues. ENA-78 stimulates neutrophils, induces chemotaxis, a rise in intracellular-free Ca^+ and exocytosis. Cross desensitization experiments indicate that ENA-78 acts through the same type of receptors as IL-8, NAP-2 and GRO-α. Thus, in accordance with the present invention, we will be able to synthesize agonists for ENA-78, including a peptide analog that binds to the IL-8 receptor.

EXAMPLE XI

Recombinant human IL-8 was obtained from Bachem and radioiodinated with IODOGEN, (Pierce, IL). Following purification by column chromatography, the radioligand was used in competitive binding assays using non-radiolabeled peptide analogs of the present invention. Analogs with the following sequences were synthesized and used as competitors. Parallel competitive binding studies were performed with the same analogs using another radiolabeled chemokine, Gro-α. The analogs used for this Example include Analogs 12 and 23-25, Sequence

I.D. Nos. 12, 23, 24, 25) and were synthesized according to procedures set forth in Example 1.

Analog 12 (Sequence I.D. No. 12):
Derived from IL-8 (this analog is sometimes referred to as "XXR")

Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 23 (Sequence I.D. No. 23):
Derived from IL-8 (this analog is sometimes referred to herein as "CVC")

Arg-Cys-Val-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 24 (Sequence I.D. No. 24):
Derived from Il-8 (this analog is sometimes referred to herein as "CMC")

Arg-Cys-Met-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys

Analog 25 (Sequence I.D. No. 25):
Derived from IL-8 (this analog is sometimes referred to as "FH")

Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His

The data in Figure 13 demonstrate the ability of the above-listed IL-8 peptide analogs to compete with radiolabeled native IL-8 for binding to the IL-8 receptor. Certain analogs displace radiolabeled ligand more efficiently than others. The specific binding of radiolabeled IL-8 is reduced to less than 50% in the presence of 10^{-4}M CMC (Figure 13C). CVC demonstrates a similar displacement curve (Figure 13B). 50% of radiolabeled IL-8 is displaced in the presence of 10^{-3} M XXR analog. The FH analog competes only slightly for binding to the IL-8 receptor. In fact, 50% displacement of radiolabeled IL-8 is not observed at the concentrations of competitor assayed.

Parallel studies were performed using radiolabeled Gro-α. As in competition studies with IL-8, the data show that certain analogs compete more efficiently than others for binding. As before, CVC and CMC compete most efficiently, displacing 50% of bound radiolabeled Gro α at approximately 10^{-5} M (Figure 14, B & C). FH (Figure 14 D) competes slightly more efficiently than XXR (Figure 14 A) with 50% of radiolabeled Gro-α being displaced at approximately 10^{-4} M as opposed to 10^{-3} M.

The Ki's were calculated using Prism and data pooled from multiple studies using percent inhibition. The results are shown in Table 5.

Table 5

	IL-8	Gro-α
IL-8	6.94×10^{-10}	9.27×10^{-10}
Gro-α	6.67×10^{-10}	4.02×10^{-11}
XXR (Analog 21)	6.67×10^{-4}	1.42×10^{-4}
CVC (Analog 22)	8.80×10^{-5}	2.07×10^{-6}
CMC (Analog 23)	6.76×10^{-5}	1.59×10^{-6}
FH (Analog 24)	4.50×10^{-4}	7.50×10^{-5}

EXAMPLE XII

The agonistic peptide analogs of the invention can act as immunostimulants in a manner similar to naturally occurring alpha chemokines, and may be useful as a protective agent for the immune system during chemotherapy and radiation therapy. Experiments were performed to determine if treatment of animals with Analog 1 could confer protection from myelosuppressive doses of gamma irradiation.

The animals used for the study were C57BL/6 females, which were obtained from Jackson Laboratories (age 8 weeks and weight 15-18g). The mice were housed in conventional cages, fed pelleted food and given water *ad libitum*.

For the experiments, the mice were administered Analog 1 continuously at a rate of 1 µl/mouse over a 24-hour period. During that time they were administered a dosage of 700 rads of gamma radiation from a cobalt source. Mice that received the Analog 1 treatment exhibited accelerated myeloid restoration following irradiation, as compared to mice that received a treatment of excipient only.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that this disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A peptide less than about 30 amino acids in length, having agonistic activity for alpha chemokines, said peptide comprising an amino acid sequence having the formula:

Glu-Leu-Arg-Cys-Xaa_1-Cys-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Xaa_7-Xaa_8-Xaa_9-Xaa_{10}-Xaa_{11}-Xaa_{12}

wherein

Xaa_1 is Gln, Met, or Val;
Xaa_2 is Ile, or Val;
Xaa_3 is Lys, Gln, or Ser;
Xaa_4 is Thr, or Ile;
Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;
Xaa_6 is Ser, Gln, Thr, or Ala;
Xaa_7 is Lys, Arg, or His;
Xaa_8 is absent or is Pro, Phe, or Gly;
Xaa_9 is absent or is Phe, Ile, or Val;
Xaa_{10} is absent or is His, Lys, or Arg;
Xaa_{11} is absent or is Pro, Leu, or Phe; and
Xaa_{12} is absent or is Lys, His or Arg.

2. The peptide of claim 1, wherein one cysteine is substituted by an amino acid selected from the group consisting of alpha aminobutyric acid, homocysteine and diaminosuberic acid.

3. The peptide of claim 1, wherein both cysteines are substituted by an amino acid selected from the group consisting of aminobutyric acid, homocysteine and diaminosuberic acid.

4. The peptide of claim 1, wherein said peptide comprises a sequence selected from the group consisting of:

Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys (Sequence I.D. No. 1);
Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe (Sequence I.D. No. 2);
Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Pro-Lys (Sequence I.D. No. 3);
Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Leu-Lys (Sequence I.D. No. 4);
Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Met-Thr-Gly-Val-His-Leu-Lys (Sequence I.D. No. 5);
Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-His-Leu-Lys (Sequence I.D. No. 6);
Glu-Leu-Arg-Cys-Met-Cys-Ile-Lys-Thr-Thr-Ser-Gly-Ile-His-Pro-Lys (Sequence I.D. No. 7);
Glu-Leu-Arg-Cys-Gln-Cys-Ile-Ser-Thr-His-Ser-Lys-Phe-Ile-His-Pro-Lys (Sequence I.D. No. 8);
Glu-Leu-Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-His (Sequence I.D. No. 9);
Glu-Leu-Arg-Cys-Gln-Cys-Leu-Gln-Thr-Val-Ala-Gly-Ile-His-Phe-Lys (Sequence I.D. No. 10); and
Glu-Leu-Arg-Cys-Val-Cys-Leu-Gln-Thr-Thr-Gln-Gly-Val-His-Pro-Lys (Sequence I.D. No. 11).

5. The peptide of claim 1, wherein said amino acid sequence further comprises an N-terminal extension having the sequence Ser-Ala-Lys.

6. The peptide of claim 1, wherein said amino acid sequence further comprises a C-terminal extension of between one and ten amino acids.

7. A peptide less than 30 amino acids in length, having agonistic activity for alpha chemokines, said peptide comprising an amino acid sequence substantially equivalent to Sequence I.D. No. 26, starting at residue 4 and continuing to residue 17.

8. The peptide of claim 7, which further comprises residues 18, 19 and 20 of Sequence I.D. No. 1.

9. A pharmaceutical composition comprising the peptide of claim 1 combined with a pharmaceutically acceptable carrier.

10. The pharmaceutical composition of claim 9, which further comprises at least one other ingredient effective for treatment of a pathological condition.

11. The pharmaceutical composition of claim 10, wherein said ingredient is selected from the group consisting of antibiotics, antivirals, antifungals, antiparasitics and anti-neoplastics.

12. A method for the treatment of a pathological condition selected from the group consisting of viral infection, bacterial infection, fungal infections, yeast infection, parasitic infection, arthritis, leukemia and solid tumor including both primary and metastatic diseases, said method comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 9, 10 or 11.

13. A peptide less than about 30 amino acids in length, having antagonistic activity for alpha chemokines, said peptide comprising an amino acid sequence having the formula:

$$Arg\text{-}Cys\text{-}Xaa_1\text{-}Cys\text{-}Xaa_2\text{-}Xaa_3\text{-}Xaa_4\text{-}Xaa_5\text{-}Xaa_6\text{-}Xaa_7\text{-}Xaa_8\text{-}Xaa_9\text{-}Xaa_{10}\text{-}Xaa_{11}\text{-}Xaa_{12}$$

wherein

Xaa_1 is Gln, Met, or Val;

Xaa_2 is Ile, or Val;

Xaa_3 is Lys, Gln, or Ser;

Xaa_4 is Thr, or Ile;
Xaa_5 is Tyr, Leu, Met, His, Val, or Thr;
Xaa_6 is Ser, Gln, Thr, or Ala;
Xaa_7 is Lys, Arg, or His;
Xaa_8 is absent or is Pro, Phe, or Gly;
Xaa_9 is absent or is Phe, Ile, or Val;
Xaa_{10} is absent or is His, Lys, or Arg;
Xaa_{11} is absent or is Pro, Leu, or Phe; and
Xaa_{12} is absent or is Lys, His or Arg.

14. The peptide of claim 13, wherein one cysteine is substituted by an amino acid selected from the group consisting of alpha aminobutyric acid, homocysteine and diaminosuberic acid.

15. The peptide of claim 13, wherein both cysteines are substituted by an amino acid selected from the group consisting of aminobutyric acid, homocysteine and diaminosuberic acid.

16. The peptide of claim 13, wherein said peptide comprises a sequence selected from the group consisting of:
Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys (Sequence I.D. No. 12);
Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe (Sequence I.D. No. 13);
Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Pro-Lys (Sequence I.D. No. 14);
Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-Ile-His-Leu-Lys (Sequence I.D. No. 15);
Arg-Cys-Gln-Cys-Leu-Gln-Thr-Met-Thr-Gly-Val-His-Leu-Lys (Sequence I.D. No. 16);
Arg-Cys-Gln-Cys-Leu-Gln-Thr-Leu-Gln-Gly-His-Leu-Lys (Sequence I.D. No. 17);
Arg-Cys-Met-Cys-Ile-Lys-Thr-Thr-Ser-Gly-Ile-His-Pro-Lys (Sequence I.D. No. 18);

Arg-Cys-Gln-Cys-Ile-Ser-Thr-His-Ser-Lys-Phe-Ile-His-Pro-Lys (Sequence I.D. No. 19);
Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-His (Sequence I.D. No. 20);
Arg-Cys-Gln-Cys-Leu-Gln-Thr-Val-Ala-Gly-Ile-His-Phe-Lys (Sequence I.D. No. 21);
Arg-Cys-Val-Cys-Leu-Gln-Thr-Thr-Gln-Gly-Val-His-Pro-Lys (Sequence I.D. No. 22);
Arg-Cys-Val-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys (Sequence I.D. No. 23);
Arg-Cys-Met-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His-Pro-Lys (Sequence I.D. No. 24); and
Arg-Cys-Gln-Cys-Ile-Lys-Thr-Tyr-Ser-Lys-Pro-Phe-His (Sequence I.D. No. 25).

17. The peptide of claim 13, wherein said amino acid sequence further comprises an N-terminal extension of between one and two non-polar amino acids.

18. The peptide of claim 13, wherein said amino acid sequence further comprises a C-terminal extension of between one and ten amino acids.

19. A peptide less than 30 amino acids in length, having antagonistic activity for alpha chemokines, said peptide comprising an amino acid sequence substantially equivalent to Sequence I.D. No. 26, starting at residue 6 and continuing to residue 17.

20. The peptide of claim 19, which further comprises residues 18, 19 and 20 of Sequence I.D. No. 26.

21. A pharmaceutical composition comprising the peptide of claim 13 combined with a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of claim 21, which further comprises at least one other ingredient effective for treatment of a pathological condition.

23. The pharmaceutical composition of claim 22, wherein said ingredient is selected from the group consisting of NSAIDS, sterum, cytotoxic drugs, cyclosporins, anti-inflammatory agents, antiarthritics and anti-autoimmune agents.

24. A method for the treatment of a pathological condition selected from the group consisting of acute and chronic inflammation, auto-immune disease, arthritis, IBD, SLE, psoriasis and reperfusion injury, said method comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 21, 22 or 23.

Fig. 1A

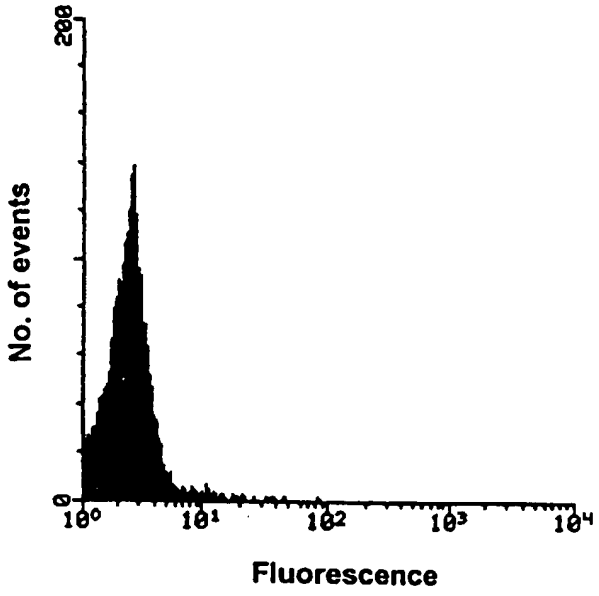

Fig. 1B

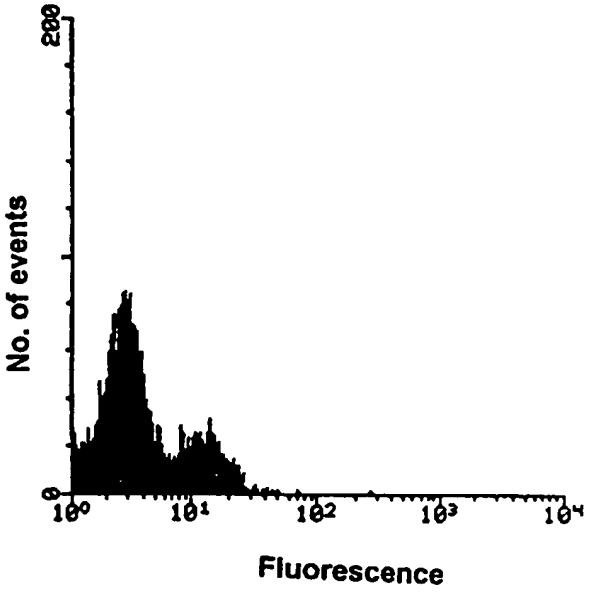

Figure 1

Fig. 2A

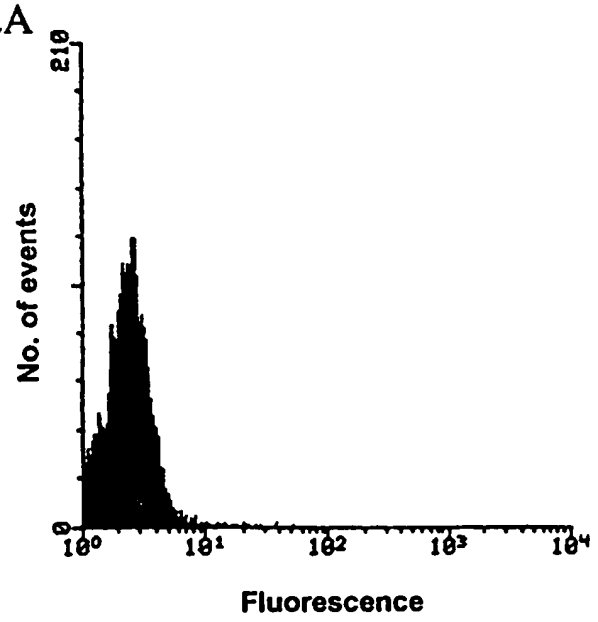

Fig. 2B

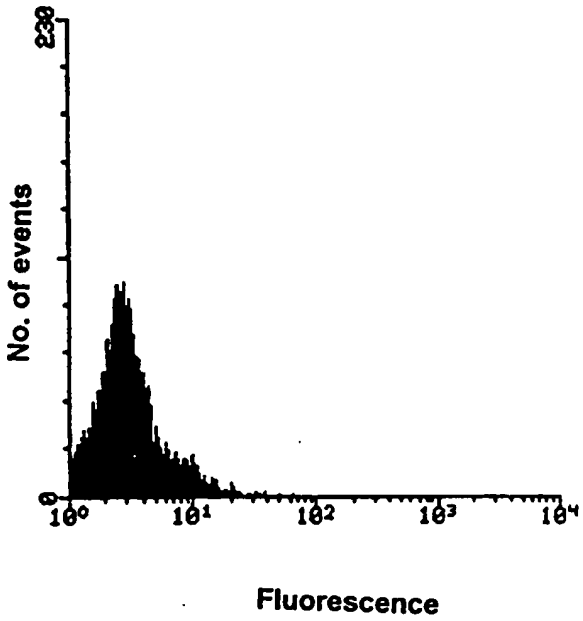

Figure 2

Fig. 3A

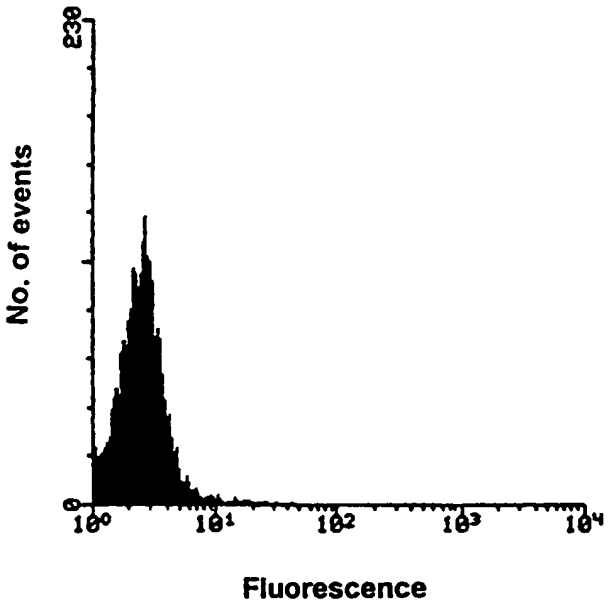

Fig. 3B

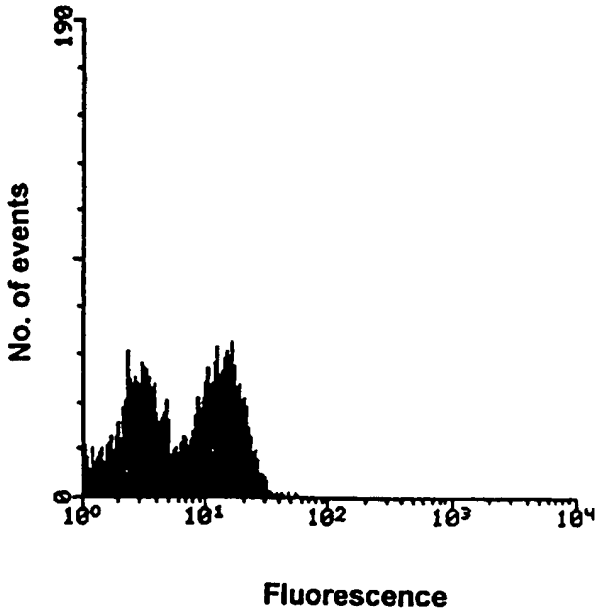

Figure 3

Fig. 4A

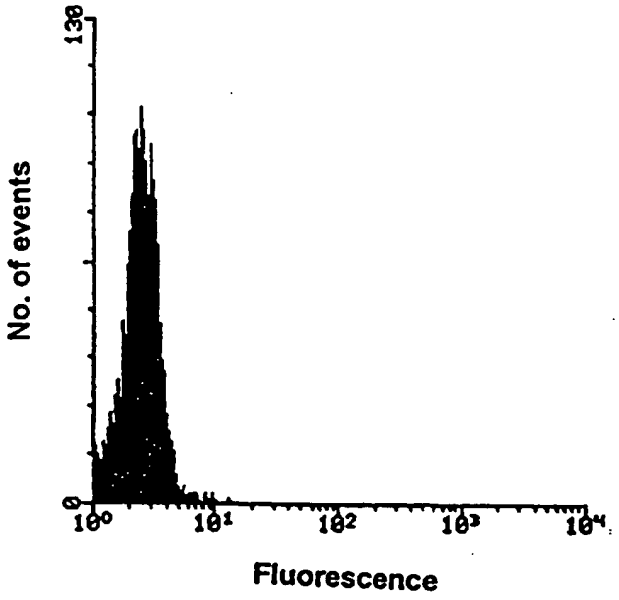

Fig. 4B

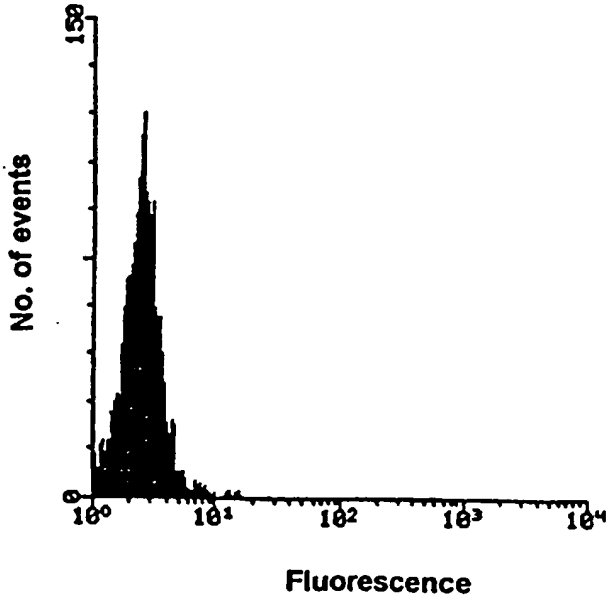

Figure 4

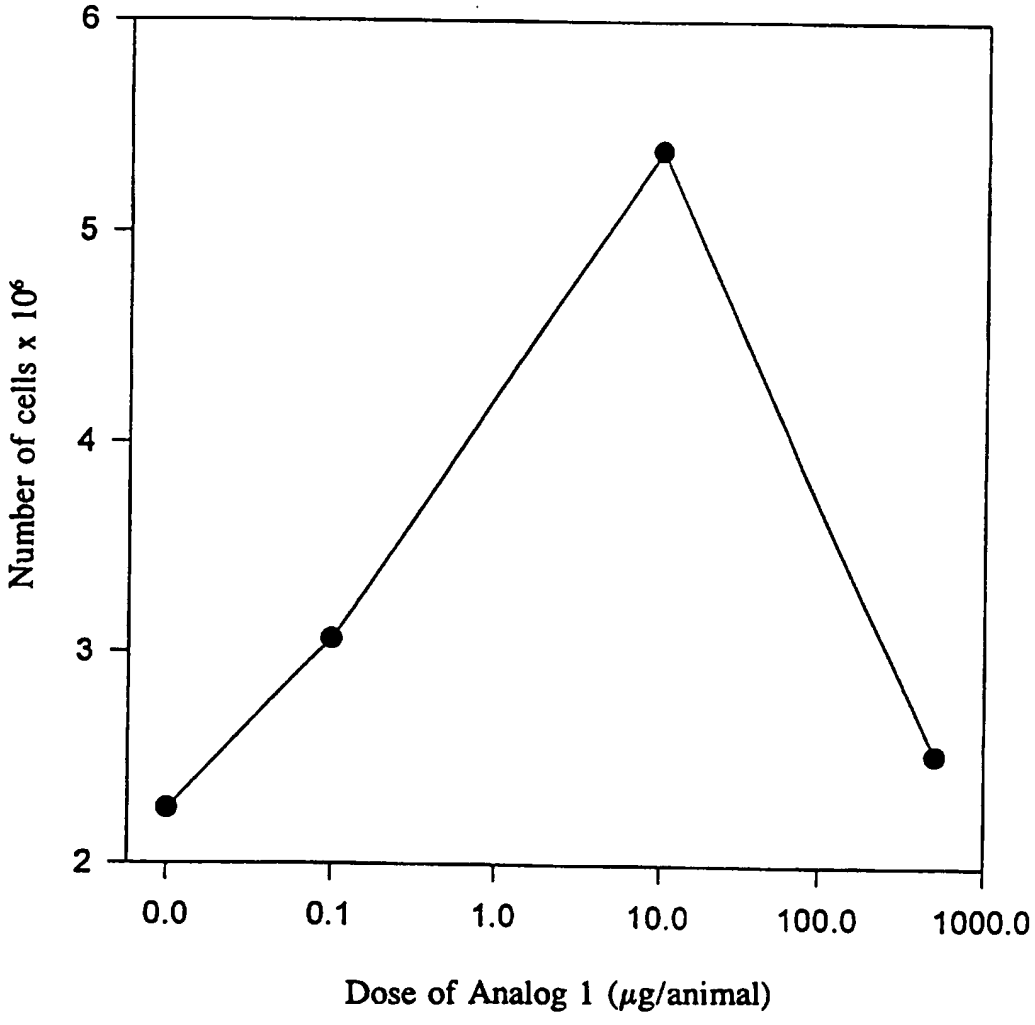
6
5
4
3
2
Number of cells x 10^6
0.0
0.1
1.0
10.0
100.0
1000.0
Dose of Analog 1 (μg/animal)

Figure 5

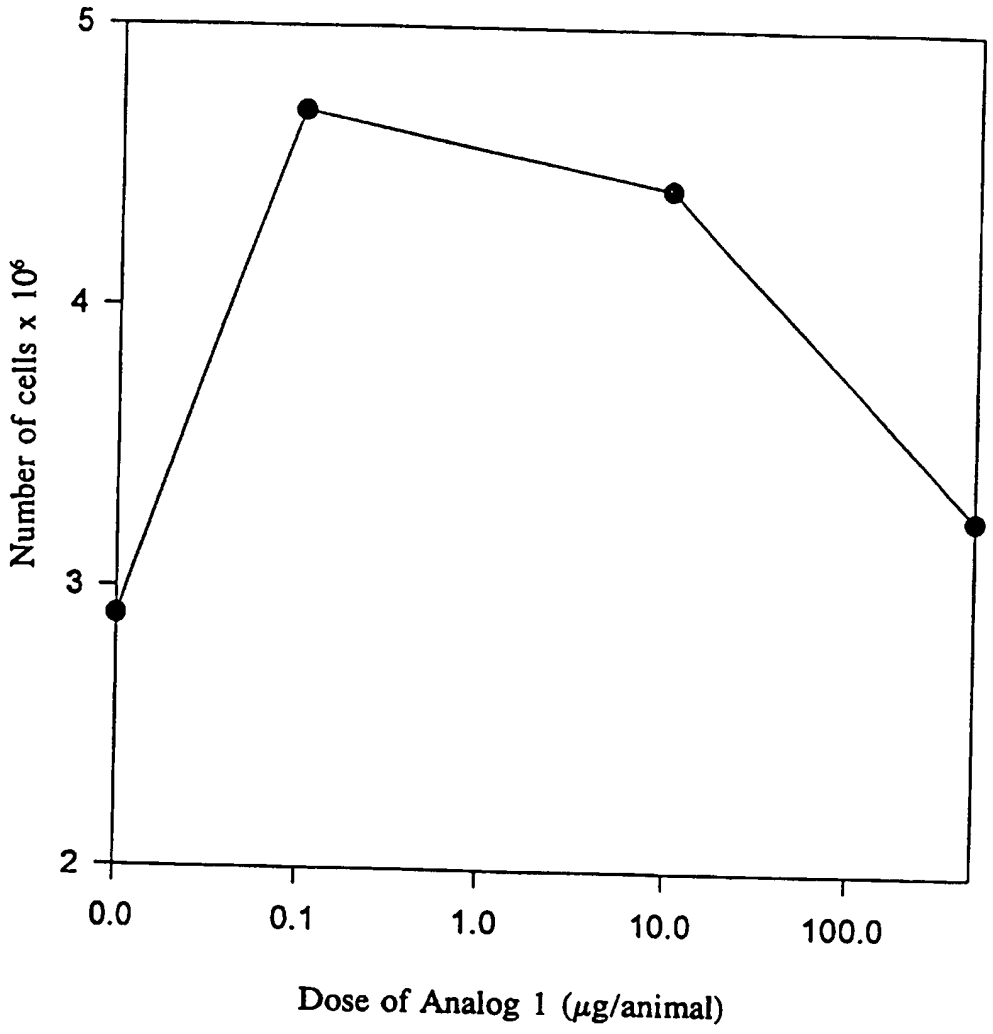
5
4
3
2
Number of cells x 10^6
0.0
0.1
1.0
10.0
100.0
Dose of Analog 1 (μg/animal)

Figure 6

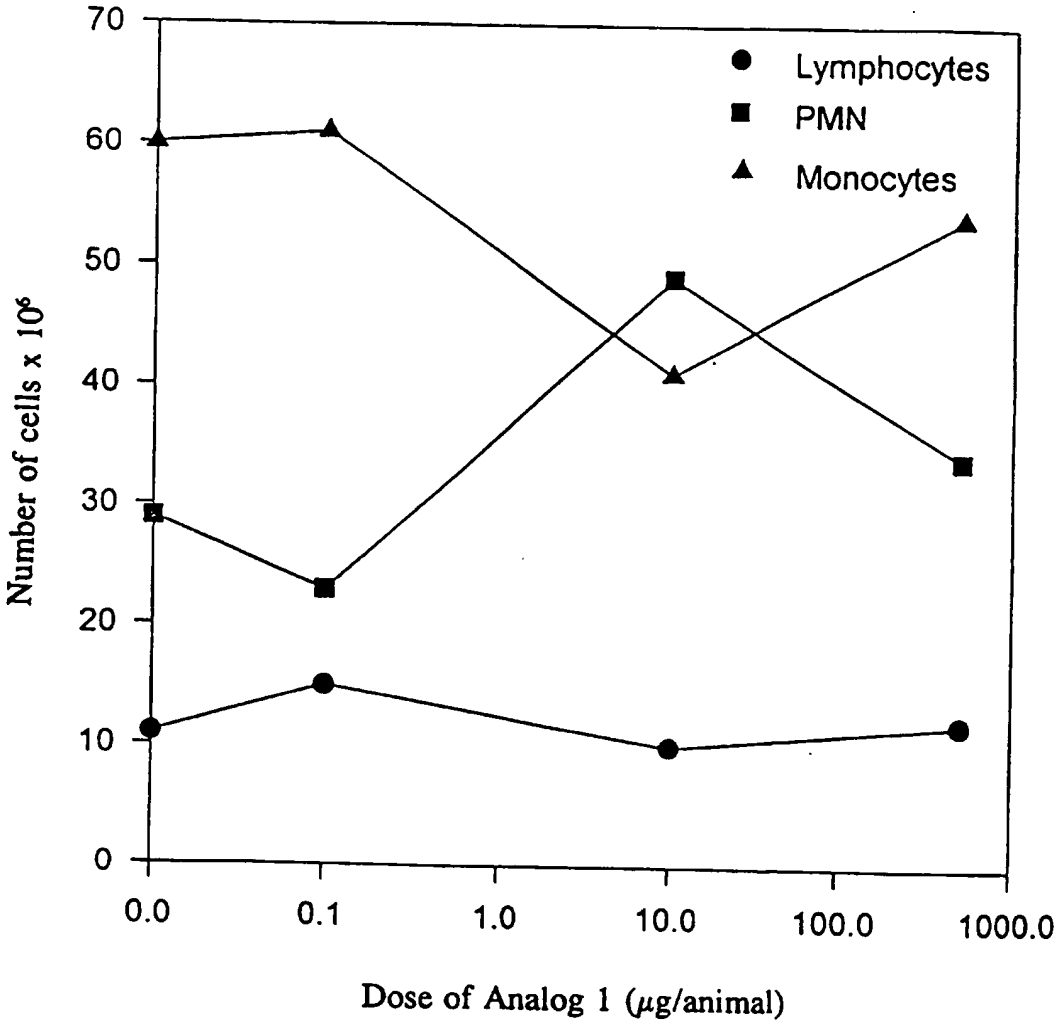
70
60
50
40
30
20
10
0
Number of cells x 10^6
Lymphocytes
PMN
Monocytes
0.0
0.1
1.0
10.0
100.0
1000.0
Dose of Analog 1 (μg/animal)

Figure 7

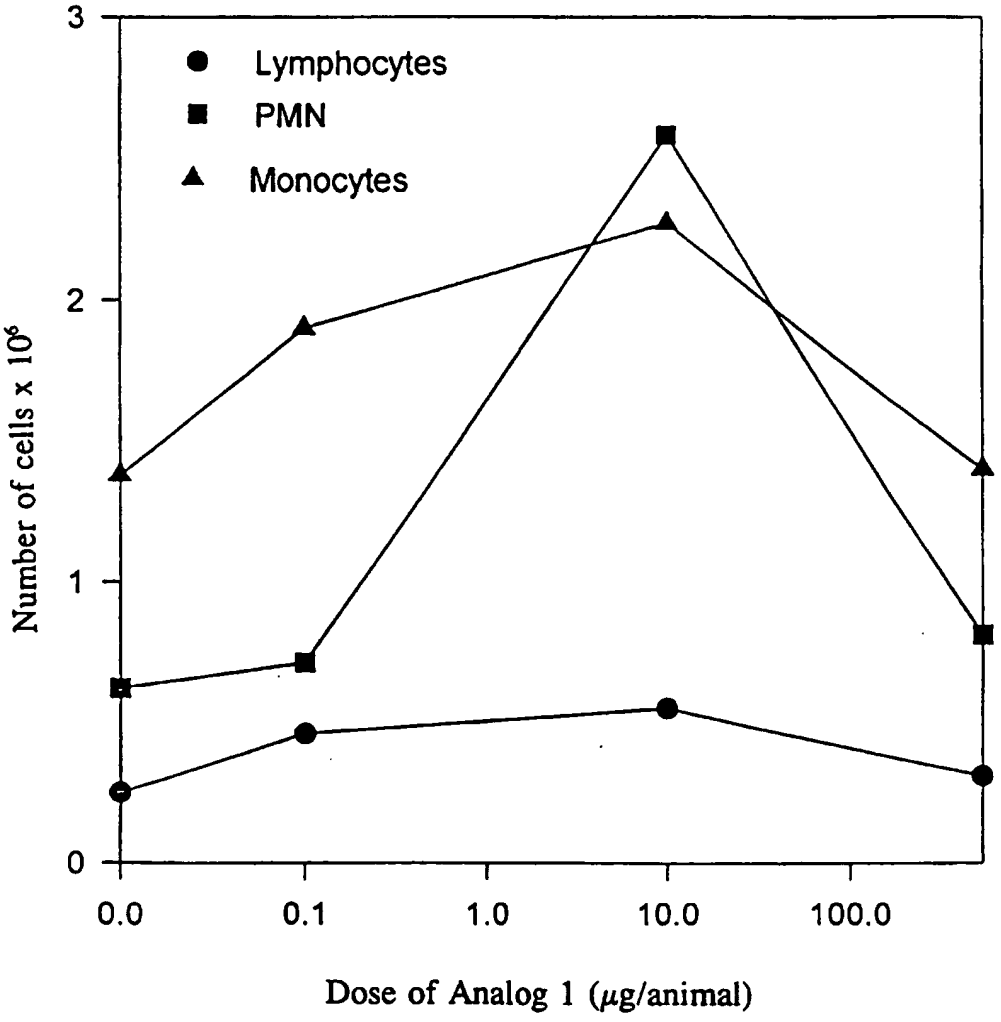
Lymphocytes
PMN
Monocytes
Number of cells x 10^6
3
2
1
0
0.0
0.1
1.0
10.0
100.0
Dose of Analog 1 (μg/animal)

Figure 8

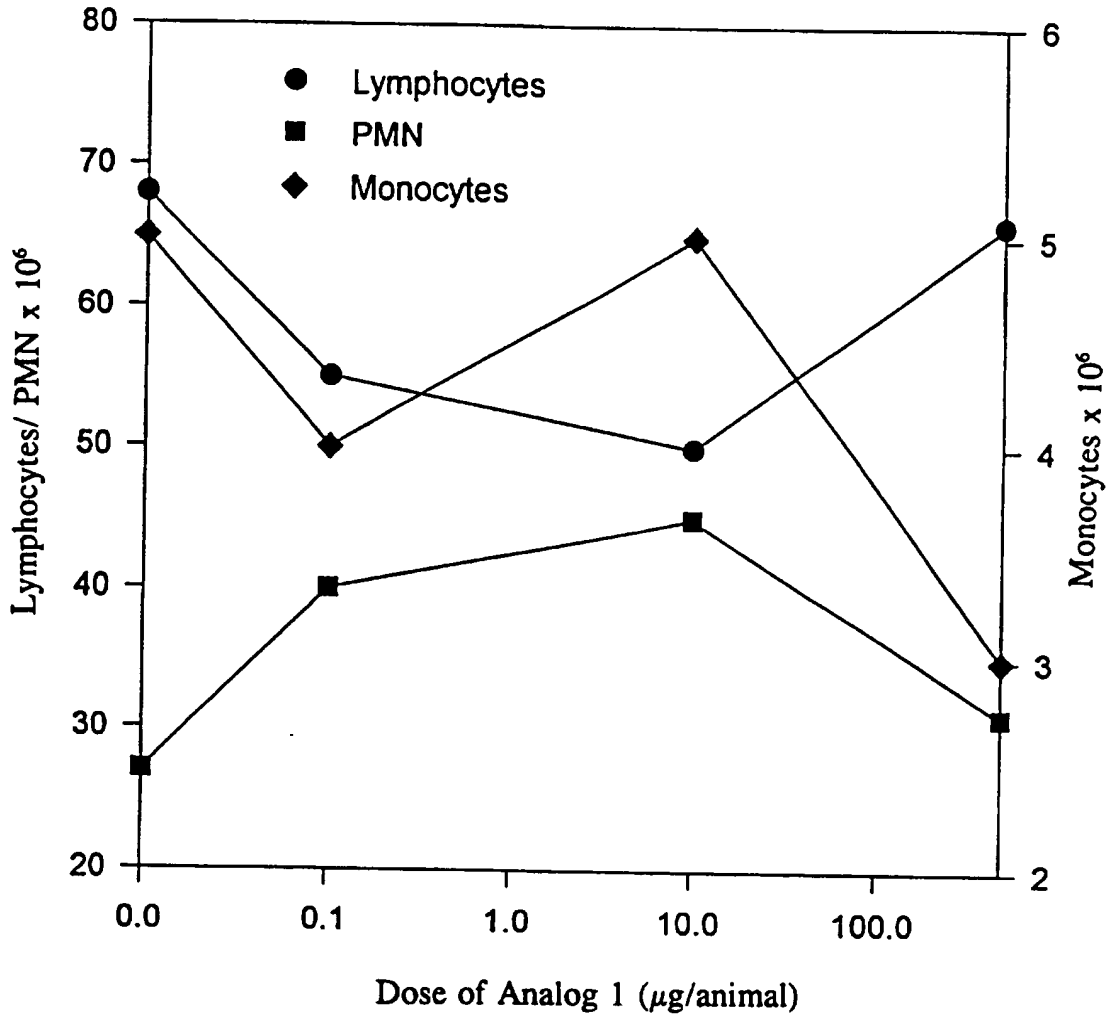
Lymphocytes
PMN
Monocytes
Lymphocytes/ PMN x 10⁶
Monocytes x 10⁶
80
70
60
50
40
30
20
6
5
4
3
2
0.0
0.1
1.0
10.0
100.0
Dose of Analog 1 (μg/animal)

Figure 9

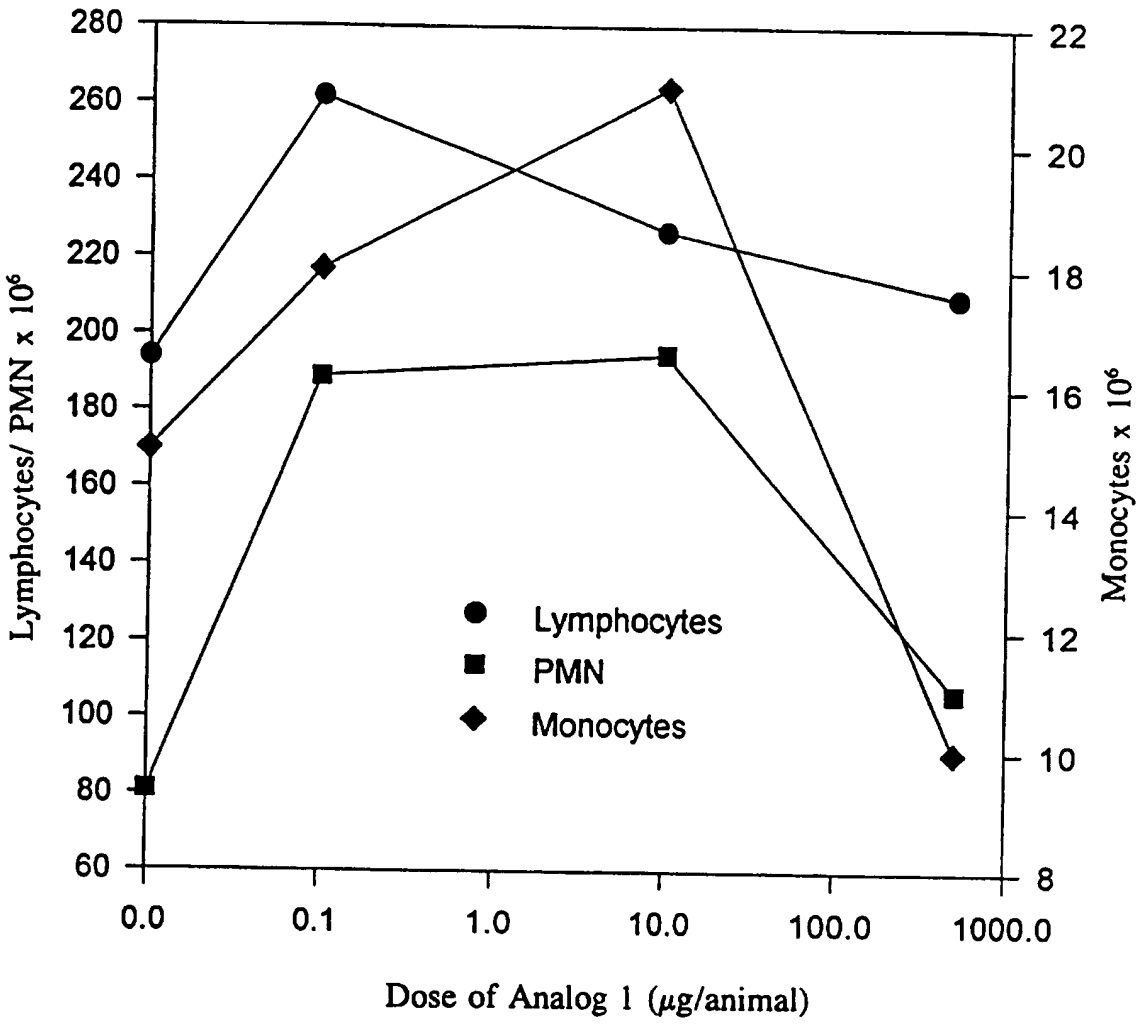
280
260
240
220
200
180
160
140
120
100
80
60
22
20
18
16
14
12
10
8
Lymphocytes/ PMN x 10⁶
Monocytes x 10⁶
Lymphocytes
PMN
Monocytes
0.0
0.1
1.0
10.0
100.0
1000.0
Dose of Analog 1 (μg/animal)

Figure 10

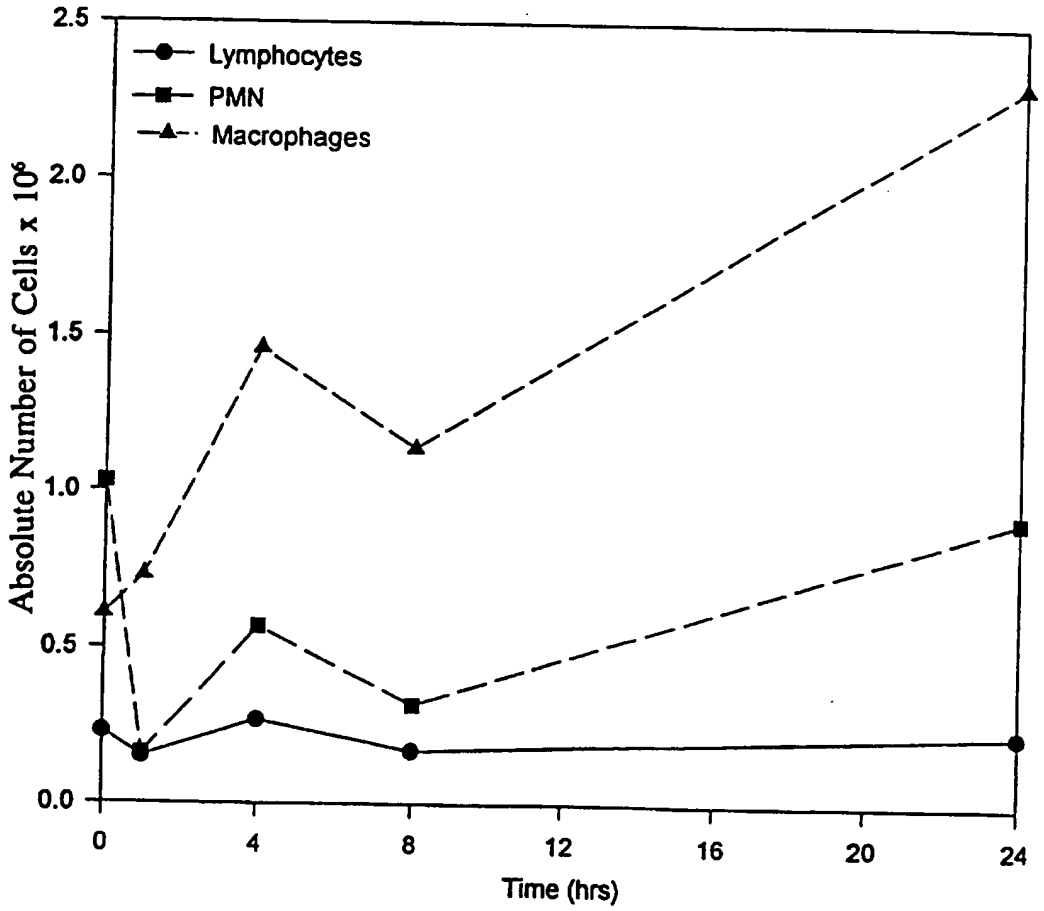
Lymphocytes
PMN
Macrophages
Absolute Number of Cells x 10⁶
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
Time (hrs)

Figure 11

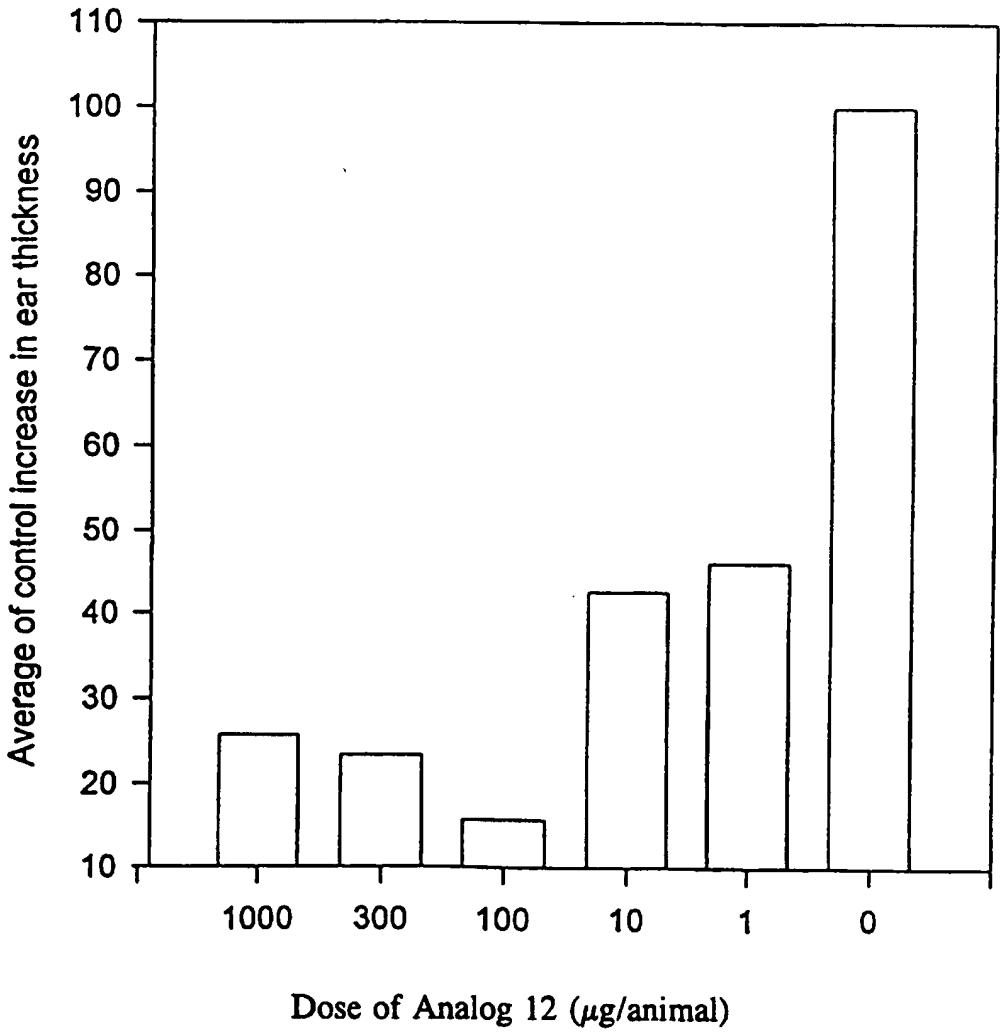
110
100
90
80
70
60
50
40
30
20
10
Average of control increase in ear thickness
1000
300
100
10
1
0
Dose of Analog 12 (μg/animal)

Figure 12

Fig. 13A

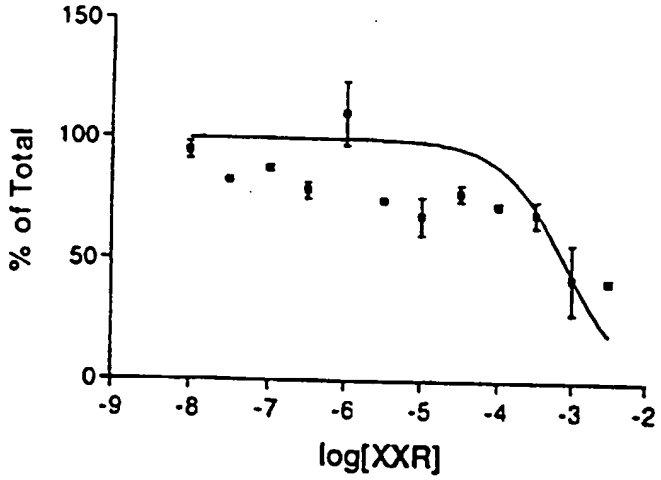

Fig. 13B

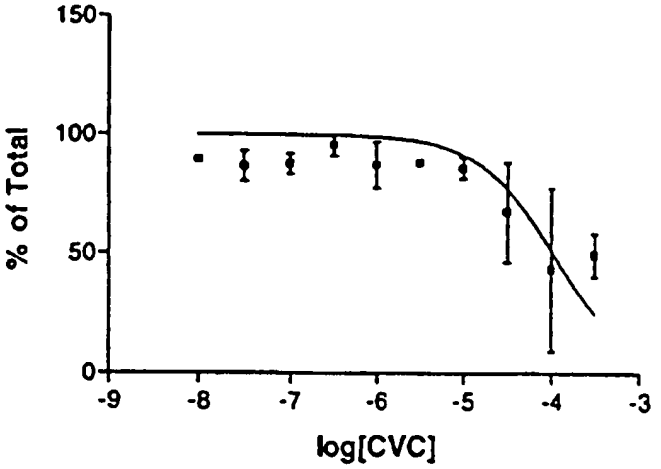

Figure 13

Fig. 13C

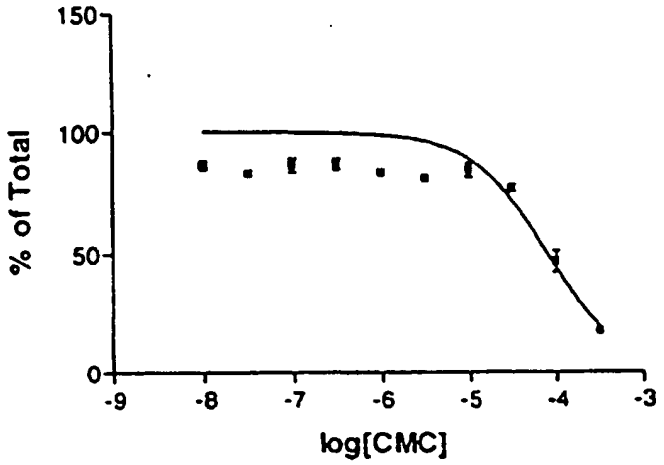

Fig. 13D

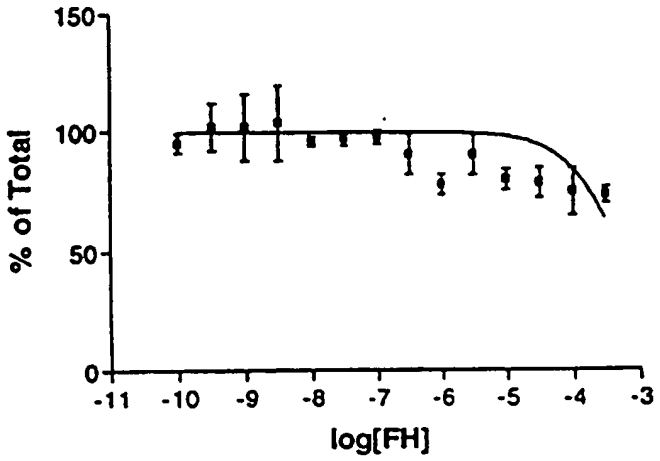

Figure 13

Fig. 14A

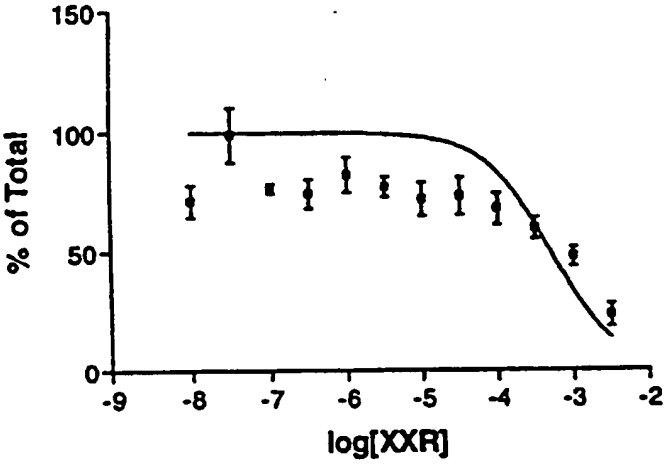

Fig. 14B

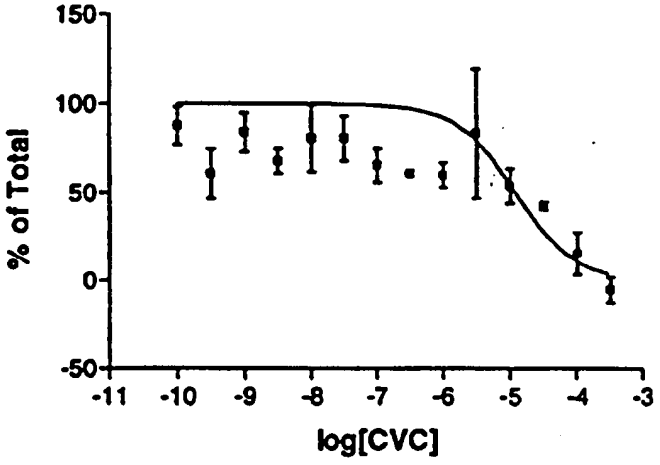

Figure 14

Fig. 14C

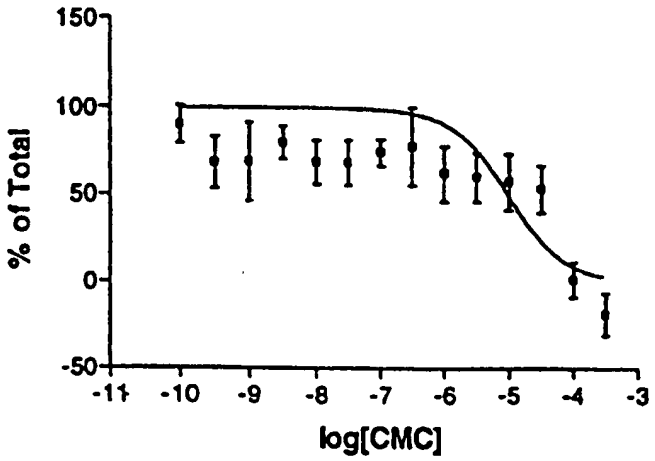

Fig. 14D

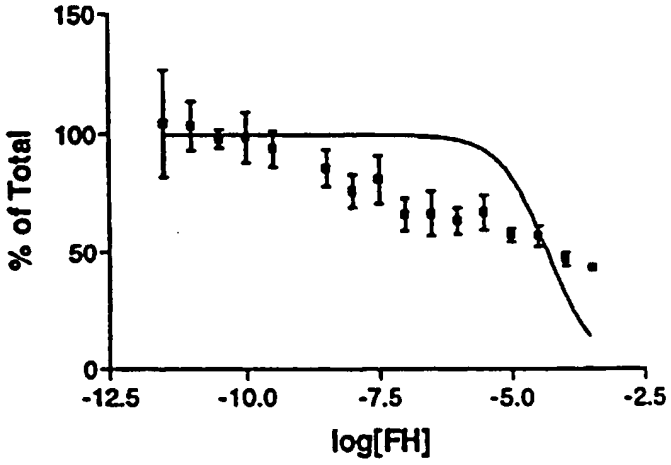

Figure 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12099

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/10, 38/16, 38/20; C07K 7/08, 14/00, 14/54
US CL :424/85.2; 514/12, 13, 14; 530/324, 325, 326, 351
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.1, 85.2; 514/12, 13, 14; 530/324, 325, 326, 327, 351; 930/140, 141

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, STN, GENESEQ, PIR, SWISS-PROT
search terms: interleukin 8, agonist, antagonist, sequences of claims 1 and 13

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 92/04372 (THE SCRIPPS RESEARCH INSTITUTE) 19 March 1992, see page 50, lines 32-35.	1, 4, 7-10, 13,16,19-22
X	US, A, 5,079,228 (COHEN ET AL) 07 January 1992, see column 2, lines 61-64, and column 7, lines 44-54.	1, 4, 6-10, 13,16,18-24
Y	US, A, 5,234,911 (CHRISTOPHERS ET AL) 10 August 1993, see column 2, lines 5-29.	22-24
A	WO, A, 93/11159 (THE BIOMEDICAL RESEARCH CENTRE LIMITED) 10 June 1993, see page 9, lines 27-32.	1-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 28 NOVEMBER 1995	Date of mailing of the international search report 29 JAN 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEFFREY E. RUSSEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12099

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	The Journal Of Biological Chemistry, Volume 269, Number 23, issued 10 June 1994, Clark-Lewis et al, "Structural Requirements for Interleukin-8 Function Identified by Design of Analogs and CXC Chemokine Hybrids", pages 16075-16081, see the abstract.	1, 4